OFFICIAL AND TENTATIVE

# METHODS OF ANALYSIS

OF THE

## ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

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# METHODS OF ANALYSIS
# A. O. A. C.

OFFICIAL AND TENTATIVE

# METHODS OF ANALYSIS

OF THE

# ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS
W. W. SKINNER (Chairman), J. A. LECLERC, L. E. WARREN,
J. W. SALE, G. G. FRARY, and MARIAN E. LAPP

THIRD EDITION, 1930

PUBLISHED BY THE
ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS
AT WASHINGTON, D.C.

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ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

The methods of the association were also copyrighted in 1916, when they were published in the Journal of the Association of Official Agricultural Chemists.

# PREFACE TO THIRD EDITION

The third issue of *Methods of Analysis* or *Book of Methods*, which are the abbreviated names of this publication, is offered to the members of the Association of Official Agricultural Chemists and to the public with a confidence that it will be as favorably received as were the previous editions. That this book of methods as originally conceived and executed fulfills the aims of its sponsors and meets the needs of a large group of official and control chemists is evidenced by the continued and unexpected demand which exhausted—six months before this edition was ready for distribution—the second edition of five thousand copies. The functions of the book and the philosophy of the development of the methods have been stated in the prefaces to former editions. These prefaces are reprinted here for reference purposes.

Although the arrangement is similar to that of the first and second editions, the changes, the rearrangements of data, and the additions and deletions are worthy of mention. The subject matter is broadly grouped into two divisions, non-foods and foods. The first division includes chapters on Soils, Fertilizers, Liming Materials, Insecticides, etc., thus bringing together related subjects, while the later chapters, devoted to foods, are arranged in alphabetical order, e.g., Baking Powders, Beverages, Beers, Coffee, Cereal Products, etc. This permits of easy reference and better meets the needs of a laboratory handbook. Several independent chapters in former editions have been combined with chapters on related subjects, for example, the chapter on Vinegars has been combined with that on Spices and Other Condiments and the chapter on Gelatine has been included under Meat and Meat Products. Beers, Wines and Distilled Liquors have been combined in one chapter. The title Feeding Stuffs has been changed to Grain and Stock Feeds.

An evidence of the progressive development of the work of the A.O.A.C. is the inclusion of new chapters on Caustic Poisons, Naval Stores, Paints, Radioactivity, and Eggs and Egg Products. Chapter headings have been assigned to such subjects as Sewage, Fibers, Paper and Paper Materials, Nuts and Nut Products, etc., indicating new lines of work contemplated by the association. Most notable among these new subjects are Vitamines, Microchemical Methods, and Bacteriological Methods. The inclusion of these chapter headings, especially the last-named, may seem out of place, but the interests of the agricultural chemist have so broadened in recent years that he finds it necessary to be professionally equipped to deal effectively with all those matters which come within the purview of research and control chemists. Therefore it has seemed wise to provide an arrangement to meet future needs. Indeed, the concepts of the profession of chemistry, especially as they apply to activities of the official chemist, have of necessity been extended to include physics, microbiology, bacteriology, microscopy, engineering, public health, etc.

An innovation in this edition is the placing of chapter numbers on each side of the page at the top and the page number at the bottom. This arrangement has facilitated editing by making it possible to include all cross references in the manuscript.

As heretofore, the methods are classified as official and tentative. In addition, note is made of those tentative methods which have received first action as official. Second favorable action by the association is necessary, however, before these methods are finally adopted as official. This classification is important to those who use these methods to support action before the courts, since they are accredited by the Secretary of Agriculture in law enforcement work and are also accepted by the States in regulatory activities. In this connection it should be understood that the methods given under each caption apply in general only to the materials mentioned therein.

That the third edition might not be unduly large owing to the volume of added material, and to make it possible to sell the book at the former price, the editors have resorted to abbreviations and contractions, and to certain forms of simplified spelling, as for example, cc for cubic centimeter, g for gram, soln for solution, temp. for temperature, m.p. for melting point, c.p. for chemically pure, and the elements and common chemicals are expressed by symbols or formulas, as Cl, Br, Zn, HCl, $CaCl_2$, $KMnO_4$, etc. In referring to the common acids and to ammonia the words "strong" and "concentrated" have been eliminated, it being understood that unless otherwise noted these reagents are the full strength product. The letter "C" after degrees of temperature has been omitted, because unless otherwise stated all temperatures in this volume are expressed in degrees centigrade. All tables have been rechecked, new ones inserted, and many of the old tables have been reduced in size when by so doing their usefulness was not impaired. These changes have saved space equivalent to many pages of printed matter.

While logically no part of *Methods of Analysis*, the editors, by a vote of the association, have included as an appendix the Definitions for Fertilizers.

The organization for the work of the third edition differed materially from that of the first and second editions. Upon the recommendation of R. E. Doolittle, Chairman of the Committee on Revision for both the first and second editions, the Executive Committee ordered the preparation and printing in *The Journal* and in separates of all changes that had been made in the methods in any one year. The purpose of this plan was to facilitate the compilation of these changes at the time of revision.

The Editorial Committee appointed by the association for the third edition consists of W. W. Skinner, Chairman; J. A. LeClerc, J. W. Sale, L. E. Warren, G. G. Frary and Marian E. Lapp. The cheerful and generous help given to the committee by the various general referees, associate referees and former referees has made this revision possible. Among those who deserve special mention are the following: S. Alfend, C. H. Badger, E. M. Bailey, L. H. Bailey, R. T. Balch, G. L. Bidwell, V. B. Bonney, I. D. Clarke, P. A. Clifford, M. R. Coe, J. Davidson, O. L. Evenson, G. S. Fraps, W. C. Geagley, J. J. T. Graham, V. E. Grotlisch, B. G. Hartmann, A. M. Henry, J. T. Keister, R. H. Kerr, J. C. Krantz, Jr., C. F. Jablonski, G. S. Jamieson, C. S. Ladd, H. A. Lepper, W. V. Linder, H. C. Lythgoe, W. H. MacIntire, J. S. McHargue, R. M. Mehurin, A. R. Merz, V. E. Munsey, E. M. Nelson, A. E. Paul, W. H. Ross, G. C. Spencer, F. P. Veitch, H. J. Wichmann, J. B. Wilson and O. B. Winter.

The work of the committee was organized by the selection of J. A. LeClerc as administrative secretary and Marian E. Lapp as assistant. To the painstaking and diligent efforts of these two members of the Revision Committee special credit for the compilation and prompt issue of the 1930 edition is due.

W. W. Skinner
*Secretary, Association of Official*
*Agricultural Chemists, and*
*Chairman, Board of Editors*

Washington, D. C.
December 31, 1930

## PREFACE TO SECOND EDITION

The methods of the Association of Official Agricultural Chemists are unique in several respects. They are the outgrowth of continual critical collaborative trial or test participated in by a large number of workers and undertaken in order to establish the accuracy of analytical results. They are subjected to a scrutiny of phraseology to insure clarity, probably unequaled in developing any similar methods. They are formulated solely by responsible Federal and State officials acting together and thus are based on underlying principles of equity. The association has always encouraged to the utmost the cooperation of representatives of interested industries, but it has jealously reserved the final formulation of its methods to official chemists. Consequently, these methods have attained an enviable position in the fields of activity occupying the attention of the association and are accepted as authoritative in matters at issue before the courts, both Federal and State.

As a result of the collaborative investigations conducted under the referee system, deletions, additions, and revisions in the methods are constantly being made. From 1884 to 1894 the methods of analysis adopted by the association were published each year, with the secretary's report of the proceedings of the annual meeting, as a bulletin of the Division of Chemistry of the United States Department of Agriculture. In 1895 the methods, brought up to date to include the changes sanctioned by the 1895 meeting, were printed as Division of Chemistry Bulletin 46, which was later revised to incorporate the changes subsequently made at the annual meetings up to 1899. The provisional methods for the analysis of foods, authorized by the 1901 meeting, were issued in 1902 as Bureau of Chemistry Bulletin 65. In 1907 the official and provisional methods as adopted by the association up to that time were printed as Bureau of Chemistry Bulletin 107, which was revised in 1908. From 1903 until 1912 circulars giving the official changes in the methods were issued annually, soon after each meeting. In 1920 the association published the methods of analysis, revised to November 1, 1919, in book form under the title "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists."

This revision, authorized in 1922, contains all the changes in methods adopted at the 1919, 1920, 1921, 1922, and 1923 meetings, as well as the polarimetric methods for the determination of sucrose and the methods for the determination of moisture and ash in wheat flour adopted in 1924.

The general plan of the original book has been retained. The cross-references, however, are to page and section, not to chapter and section, and the following changes in the text have been made: Two new chapters, one on agricultural liming materials and one on gelatin, have been added; the chapter on water has been expanded to include brines and salts; the methods for sugar products formerly included in the chapters, Foods and Feeding Stuffs and Saccharine Products, have been combined in a chapter entitled Sugars and Sugar Products; and the methods for stock feeds have been rearranged under the chapter heading Feeding Stuffs.

The Committee on Editing Methods of Analysis of the association, consisting of R. E. Doolittle (chairman), G. W. Hoover, W. H. MacIntire, A. J. Patten, B. B. Ross, and J. W. Sale, prepared this revision. Miss Marian E. Lapp, associate editor of *The Journal* gave the committee valuable assistance and edited the manuscript. Referees, associate referees, and other members of the association assisted in compiling and critically reviewing the work.

W. W. Skinner
*Secretary, Association of Official*
*Agricultural Chemists*

January 1, 1925

# INTRODUCTION TO SECOND EDITION

In the present publication, the technic of agricultural analysis is brought up to date. The most valuable contribution made to agriculture in the last forty years has been that of the standardization of the chemical and physical methods of research in agriculture by this association. The importance of sound and accurate laboratory methods is not so highly appreciated as it should be. Such methods are of supreme value not only to the agricultural worker, but also to all research workers in every branch of science, and especially in chemistry. A striking illustration of this is seen in the work on the nature and relative weight of the atom. It is not appointed to every one to become a leading expert. There are many good artists but only a few masters. There are many notable workers in research, but only a few Curies, Ramsays, Millikans, Rutherfords, and Richards. It is as much method and delicacy of manipulation as vision and initiative that make the master.

This revision of methods improves the value of the instruments in the hands of the seeker of new facts and the explanation of new laws.

Harvey W. Wiley

# PREFACE TO FIRST EDITION

In presenting this revision of the official and tentative methods of analysis of the Association of Official Agricultural Chemists, it is appropriate to give a brief statement of the organization of the association, its purpose, and the procedure by which the methods are adopted.

Membership in the association is institutional and includes the State Departments of Agriculture, the State Agricultural Colleges and Experiment Stations, the Federal Department of Agriculture, and the Federal, State, and City offices charged with the enforcement of food, feed, drug, fertilizer, insecticide and fungicide control laws.

The association was founded at Philadelphia, Pa., September 9, 1884, by the following representative agricultural chemists of that time, the organization being the result of a series of informal meetings held the immediately preceding years:

Prof. H. W. Wiley, Chemist of the Department of Agriculture, Washington, D. C.
Mr. Clifford Richardson, Assistant Chemist of the Department of Agriculture, Washington, D. C.
Mr. Philip E. Chazal, State Chemist of Virginia.
Dr. Chas. W. Dabney, Jr., State Chemist of North Carolina.
Dr. W. J. Gascoyne, State Chemist of North Carolina.
Dr. E. H. Jenkins, Connecticut Experiment Station.
Prof. John A. Myers, State Chemist of Mississippi.
Prof. H. C. White, State Chemist of Georgia.
Mr. C. DeGhequier, Secretary National Fertilizer Association.
Dr. Schumann, Dr. Lehmann, Mr. Gaines and others.

At the first meeting methods for the determination of ammonia, phosphoric acid and potash in commercial fertilizers were adopted and work was begun for the perfection and adoption of methods for the entire range of agricultural chemistry. Later the passage of food and drug and insecticide and fungicide control legislation by the States and by the Federal Government made it necessary to extend the scope of the association's activities for reason that the association methods were designated as the official methods for the enforcement of such legislation as well as for the control of feeds and fertilizers by the various states.

To attain the aims of the association for a set of accurate methods, a system was evolved by which the methods in question are subjected to the most rigorous and painstaking scrutiny before they can be adopted. A "referee" is appointed for any subject for which the association has not yet an official method or for a method which seems to require further investigation. The referee conducts analyses according to the methods suggested for adoption in comparison with methods already established, obtaining the collaboration of as many as possible of the workers in that field. In addition, a great deal of original research has been inaugurated on new methods. This system developed logically until at the present time, in order to be adopted as "tentative," a method must be recommended to the association by the referee, and such recommendation is made only after the method has undergone a thorough collaborative and critical study. Further, the special committee on methods must approve the recommendation and the method must be accepted by a vote of the association. In order to become "official," a method must be again accepted at another annual meeting. The recommendations of referees are published in the reports of the proceedings of the association in the *Journal of the Association of Official Agricultural Chemists*, so that all tentative methods are made public before being adopted. This permits consideration and criticism by chemists who are not members of the association. It is immediately apparent that a method can be made official only after the most thorough series of tests, not alone for accuracy, but for ease of operation as well. It may be stated without reservation that more elaborate and painstaking effort has been expended on this collection of analytical methods than upon any other set of similar methods in the field of chemical science.

The compilation and revision of the methods presented in this book was made by a committee of the association, consisting of R. E. Doolittle (chairman), B. L. Hartwell, G. W. Hoover, A. F. Seeker (deceased), J. P. Street, and W. A. Withers. Later, on the resignation of J. P. Street, A. J. Patten was appointed a member of the committee and the work of revision was continued.

A preliminary revision, antedating the revision published in this book, was printed in 1916 as supplementary parts to Volumes I and II of the *Journal of the Association of Official Agricultural Chemists*. In this preliminary revision the committee received important assistance from R. L. Emerson, F. C. Blanck, and N. A. Parkinson. At that time the scheme of numbering the sections in each chapter was adopted in order to simplify the system of cross-references.

In the preparation of the present revision J. A. MacLaughlin rendered valuable assistance. Acknowledgment is also made to the Library of the Department of Agriculture for assistance.

Throughout its work, it has been the aim of the committee not only to bring the methods up to date, but especially to state the procedure with such lucidity and in such detail as to make it possible for any trained chemist to operate without being in doubt at any time.

The work of the committee has been one of critical revision, compilation and editing. The work of developing the methods was done by the various referees and their collaborators who have reported to the association at its annual meetings during the last decade. To them is due the credit for the subject matter of this book.

C. L. Alsberg
*Secretary of the Association of*
*Official Agricultural Chemists*

September 17, 1920

# INTRODUCTION TO FIRST EDITION

By Dr. Harvey W. Wiley, Honorary President of the Association of Official Agricultural Chemists

In the present edition of the methods of analysis, official and tentative, of the Association of Official Agricultural Chemists, the technic of analytical procedures has been revised to November 1, 1919. The monumental work of the Association of Official Agricultural Chemists is not only well known in the profession in this country, but is recognized in all countries as being the last word in agricultural chemical technic. The methods of determining the composition of agricultural products, as well as of all bodies related to agriculture, has been recognized also by the courts of this country. In case of judicial proceedings where different methods of analysis have been employed, the court, in all cases where the question has arisen, has recognized the official methods as binding.

At the time of the organization of this body, referred to in the Preface, agricultural methods of research, from the chemical point of view, were extremely chaotic. The progress of agricultural science which has marked its history in the last third of a century could not have been maintained amid these chaotic conditions. The methods adopted by the founders of this association for correcting this state of affairs have been shown by experience to be the best possible. I can say that the improvement in agricultural chemical technic has almost kept pace with the growth of the association.

The gradual incorporation in the membership of the association of those scientific men engaged in the control of foods and drugs has widened the scope without altering the purpose of the original founders. Today we find a body of scientific workers in agriculture and related subjects numbering quite half a thousand, who, by their activities and collaboration, have contributed to the pages of this volume, directly or indirectly. The scientific knowledge of agriculture which has been verified and extended by this association now forms the foundation of all agricultural improvement.

The profession of agriculture is the fundamental industry of this country. Everything which strengthens the foundations of this industry benefits the country at large. Our workers are not banded together for personal preferment, either in wages or in authority. They have united for the sole purpose of benefitting agriculture and thus increasing production. They have not asked for shorter hours, nor for higher pay. They have worked in season, out of season, by day and by night, on work days and holidays, to perfect that science which, in its application, is the most powerful factor in scientific agriculture.

The ability of the agricultural industry to withstand the assaults which are made upon it at the present time is largely due to the successful efforts of our association. The agricultural industry has been built upon a rock and thus it is able to withstand the winds and the floods. This industry is now in a more critical condition than any other. The allurements of the city and the high wages of labor therein, have drawn from the farm much of its best blood and energy. Congregate life has become so much more attractive than discrete life that it is hard to keep the young of both sexes upon the farm. Yet it is plain that if man power and woman power upon the farm now be depleted the industry must suffer. Making the farm attractive does not merely mean beautifying the house in which the farmer lives, making it more sanitary, planting trees, flowers and shrubs, but it means also the best knowledge of the soil and its properties; the most scientific data respecting the manufacture and use of fertilizing materials; the most accurate knowledge of the character of crops best suited to the soil, and the best system of rotation which will help develop from the soil its most generous contribution. In other words, not only must the farmer's farm be attractive and sanitary, but it must also be productive and dividend paying.

We can well imagine the worth of the work which our association has done by picturing for a moment what the present agricultural industry would be if all that our science has contributed to it were stricken from human records. In such a deplorable condition starvation would surely be staring the world in the face. In the quiet corners of the laboratory, by the midnight oil and by personal devotion, the means which enable the farmer to get more remunerative crops have been worked out and perfected. These workers, male and female, who have done this gigantic task have never been heralded in the public press, nor received encomiums of an admiring world. They have done their work silently and effectively, without expectation of praise and without hope of pecuniary reward. Their real reward has been in the consciousness of duty done. The referees who have presided over this great work for the past thirty-six years and those who have aided them in these investigations, merit the generous regard and esteem of the whole scientific world, as well as the whole agricultural world. Our association has been not one of debate nor of visionary plans of human welfare, but rather of hard work and concentrated devotion to the cause.

The volume which is now laid before you contains the very last word of all that is important in agricultural research from the chemical and physical point of view. This does not mean that the field is fully exploited. The great unknown of tomorrow doubtless holds in its secret embrace even greater prospects for human betterment than the days which have already passed. This association stands ready and with expectant breath to receive the messages of tomorrow and translate them to the agricultural world.

Washington, D. C.,
September 15, 1920

# CONTENTS

PAGE
Preface to Third Edition . . . . v
Preface to Second Edition . . . . vii
Introduction to Second Edition . . . . ix
Preface to First Edition . . . . ix
Introduction to First Edition . . . . xii
Illustrations . . . . xvi
Definition of Terms . . . . xvii

CHAPTER
I. Soils . . . . 1
II. Fertilizers . . . . 14
III. *
IV. Agricultural Liming Materials . . . . 32
V. *
VI. Insecticides and Fungicides . . . . 35
VII. Caustic Poisons . . . . 66
VIII. Naval Stores . . . . 68
IX. Paints . . . . 73
X. Leathers . . . . 92
XI. Tanning Materials . . . . 96
XII. Plants . . . . 102
XIII. *
XIV. *
XV. Baking Powder . . . . 115
XVI. Beverages (Non-alcoholic and Concentrates) . . . . 131
XVII. Beers, Wines and Distilled Liquors . . . . 134
XVIII. Coffee and Tea . . . . 150
XIX. Cacao Bean and Its Products . . . . 157
XX. Cereal Products . . . . 165
XXI. Coloring Matters in Foods . . . . 184
XXII. Dairy Products . . . . 214
XXIII. Eggs and Egg Products . . . . 244
XXIV. *
XXV. Flavoring Extracts . . . . 251
XXVI. Fruits and Fruit Products . . . . 264
XXVII. Grain and Stock Feeds . . . . 277
XXVIII. Meat and Meat Products . . . . 289
XXIX. Metals in Foods . . . . 306
XXX. *
XXXI. Oils, Fats and Waxes . . . . 314
XXXII. Preservatives and Artificial Sweeteners . . . . 334
XXXIII. Spices and Other Condiments . . . . 349
XXXIV. Sugar and Sugar Products . . . . 363
XXXV. Vegetables and Vegetable Products . . . . 397
XXXVI. *
XXXVII. Waters . . . . 403
XXXVIII. Radioactivity . . . . 431
XXXIX. Drugs . . . . 437
XL. *
XLI. *
XLII. Tables . . . . 495
Appendix . . . . 490
General Index . . . . 565

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* The subject matter of the following chapters: III, Sewage; V, Agricultural Dust; XIII, Fibers; XIV, Paper and Paper Material; XXIV, Fish and Other Marine Products; XXX, Nuts and Nut Products; XXXVI, Vitamins; XL, Bacteriological Methods; and XLI, Microchemical Methods, indicate activities which the association has begun or is planning to undertake.

# ILLUSTRATIONS

PAGE
Fig. 1. Quadruplicate Shaking Apparatus for Determination of Carbonate Carbon in Soils.......... 2
Fig. 2. Absorption Tower.......... 3
Fig. 3. Apparatus for Automatic Filtration and Measurement of Lime Solutions.......... 33
Fig. 4. Apparatus for Distillation of Arsenious Chloride.......... 36
Fig. 5. Dean and Stark Distilling Tube Receiver.......... 53
Fig. 6. Apparatus for the Determination of Fluorine by the Volatilization Method.......... 61
Fig. 7. Reaction Flask for the Determination of Fluorine.......... 62
Fig. 8. Apparatus to be Used in Volatilization Method for Determination of Mercury.......... 63
Fig. 9. Apparatus for the Distillation of Turpentine Oil Over Open Flame.. 69
Fig. 10. Apparatus for the Distillation of Turpentine Oil Over Bath.......... 70
Fig. 11. Metal Extractor Used for Extracting Tanning Materials.......... 100
Fig. 12. Suction Device Used in Micro Method for the Determination of Calcium.......... 105
Fig. 13. Knorr's Apparatus for the Determination of Carbon Dioxide.......... 115
Fig. 14. Heidenhain's Apparatus for the Determination of Carbon Dioxide.. 117
Fig. 15. Apparatus for the Gasometric Determination of Carbon Dioxide.... 119
Fig. 16. Apparatus for the Determination of Fluorine.......... 127
Fig. 17. Reaction Flask Used in the Determination of Fluorine.......... 128
Fig. 18. Apparatus for the Determination of Volatile Acids.......... 140
Fig. 19. Preparing the Dough for Panning.......... 179
Fig. 20. Apparatus for Titration with Titanium Trichloride.......... 205
Fig. 21. Melting Point Apparatus.......... 208
Fig. 22. Hortvet Cryoscope.......... 220
Fig. 23. U. S. Bureau of Standards Tested Thermometer.......... 222
Fig. 24. Photomicrographs Identifying Malted Milk and Its Allied Products. 232
Fig. 25. Photomicrographs Identifying Malted Milk and Its Allied Products. 234
Fig. 26. Apparatus for Drying Pentabromacetone by Aspiration.......... 274
Fig. 27. Apparatus Used in Method II for the Determination of Moisture... 277
Fig. 28. Apparatus for the Determination of Ammonia.......... 290
Fig. 29. Van Slyke Apparatus for the Determination of Amino Nitrogen.... 298
Fig. 30. Details of the Deaminizing Bulb and Connection.......... 299
Fig. 31. Generator to be Used with the Gutzeit Method.......... 307
Fig. 32. Apparatus for the Determination of the Melting-point.......... 318
Fig. 33. Apparatus for the Determination of the Polenske Number.......... 323
Fig. 34. Monier-Williams Apparatus for Determination of Sulfurous Acid... 345
Fig. 35. Apparatus for Determination of Formic Acid.......... 347
Fig. 36. Reaction Cylinder to be Used in the Determination of Bromide..... 426
Fig. 37. Apparatus for the Determination of Radioactivity by the Emanation Method.......... 432
Fig. 38. Apparatus for the Determination of Arsenic in Iron-Arsenic Tablets. 477

# DEFINITION OF TERMS AND EXPLANATORY NOTES

(1) The term "water" used in the methods means distilled water.

(2) The reagents listed below, unless otherwise specified, have the approximate strength stated and conform in purity with the requirements of the United States Pharmacopoeia.

| | |
|---|---|
| Sulfuric acid | Specific gravity 1.84 |
| Hydrochloric acid | Specific gravity 1.184 |
| Nitric acid | Specific gravity 1.42 |
| Fuming nitric acid | Specific gravity 1.50 |
| Glacial acetic acid | Specific gravity 1.048 (25°) |
| Hydrobromic acid | Specific gravity 1.38 |
| Phosphoric acid | 85 per cent strength by weight |
| Ammonium hydroxide | Specific gravity 0.90 |

(3) All other reagents and test solutions, unless otherwise described in the text, conform to the requirements of the United States Pharmacopoeia or of the American Chemical Society. When the anhydrous salt is intended, it is so stated; otherwise the salt referred to is the crystallized product.

(4) In the expressions (1+2), (5+4), etc., used in connection with the name of a reagent, the first numeral indicates the volume of the reagent used, and the second numeral indicates the volume of water. For example, hydrochloric acid (1+2) means a reagent prepared by mixing one volume of hydrochloric acid with two volumes of water. When one of the reagents is a solid the expression means parts by weight, the first numeral representing the solid reagent and the second numeral the water.

(5) In making up solutions of definite percentage it is understood that $x$ grams of substance is dissolved in water and made up to 100 cc. Although not theoretically correct, this procedure will not result in any appreciable error in any of the methods given in this book.

(6) For the sake of simplicity the abbreviations Cl and I instead of $Cl_2$ and $I_2$ are used for chlorine and iodine. Similar abbreviations have been used in other cases.

(7) Recommendations to delete certain official methods were approved by the association at the 1930 meeting, and first action for the deletion of such methods was taken. Before the methods are finally deleted, however, a second action must be taken. The text of such methods is not printed in this edition but may be found in the 2nd (1925) edition of *Methods of Analysis*. All official methods thus approved for deletion are marked with an asterisk unless a fuller explanatory note is given in connection with the method.

(8) All calculations are based on the table of international atomic weights, Table I, under **XLII**.

(9) The table for the conversion of specific gravity at 20/4° into alcohol is given in Methods of Analysis, A.O.A.C. 1925, page 464. Action will be taken at the 1931 meeting of the association to make 15.56/15.56°, 20/20° and 25/25° official in place of 20/4°. Wherever directions are given to determine specific gravity at 20/4°, it is advisable to use the temperatures given above. Tables for the conversion of specific gravity into alcohol at these temperatures are given in this edition.

# Official and Tentative Methods of Analysis

OF THE

# ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

## I. SOILS[1]—TENTATIVE

### 1 DIRECTIONS FOR SAMPLING

(In view of the variability in soils, it seems impossible at the present time to devise an entirely satisfactory method for sampling. It is obvious that the details of procedure should be determined by the purpose for which the sample is taken.)

Remove from the surface all vegetable material not incorporated with the soil. Take a sufficient number of samples to insure a composite sample that will be representative of the tract sampled, to the average depth of plowed soil, usually about 7 inches, and also take a composite sample from each important and distinctly different soil stratum to the depth of 40 inches, using a soil tube or auger. If a soil auger is used, before boring below the plowed depth enlarge the first boring and carefully clean out the hole to prevent contamination of the successive sub-strata while withdrawing the samples. The sampling should be done when the soil is reasonably dry. Thoroly mix the samples of each depth and dry in a well ventilated, cool place.

In order to calculate the percentage results obtained by analysis to pounds per given area of the soil, determine the weight of a given volume of the soil as it lies in the field.

### 2 PREPARATION OF SAMPLE

(**a**) Reduce any soil lumps in the air-dried soil by rubbing in a porcelain mortar or by any other equally effective method that will not reduce the rock fragments and pass thru a sieve having circular openings 1 mm in diameter. Thoroly mix the sifted material and preserve in a suitable stoppered container. Weigh and discard the detritus.

(**b**) If necessary for the determination of the total quantity of any constituent, *pulverize* more finely a sub-sample of (**a**).

If deviations from this procedure are deemed necessary, record them with the results.

### 3 MOISTURE

Dry 2 g of the sample, prepared as directed under **2** (**a**), in a wide-mouthed weighing bottle at 100° to constant weight. Report the loss in weight as percentage of the moisture-free weight of the sample taken.

### 4 LOSS ON IGNITION

(This method gives only a rough approximation of the organic matter and is not at all accurate for soils containing much combined water.)

Ignite the soil from **3** in a Pt dish or suitable substitute to full redness, stirring occasionally, until organic matter is destroyed. If the soil contains appreciable quantities of carbonates, moisten, after cooling, with a few drops of a saturated soln of

$(NH_4)_2CO_3$, dry, heat to dull redness to expel the $NH_4$ salts, cool in a desiccator, and weigh. Report the percentage loss in weight as organic matter.

## CARBONATE CARBON

### 5 APPARATUS

*Quadruplicate shaking apparatus for evolution of carbonate carbon in soils of high or low carbon content.*[2]—This apparatus (Figs. 1 and 2) consists of a horizontal holder (*H*) 21 inches long, ⅞ inch thick, and 1¾ inches wide, having properly spaced slots made to fit loosely the neck of a 300 cc. Erlenmeyer flask taking a No. 6 rubber stopper. The holder (*H*) is suspended horizontally from a bar by means of brass strips 1¾ inches wide and 24 inches long. The common intake for purification of the incoming air leads from a tube about 25 inches long.

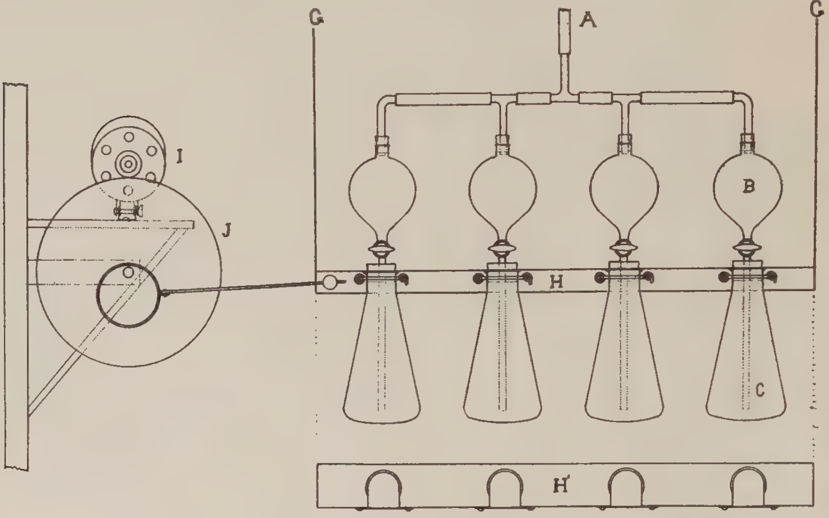

FIG. 1.—QUADRUPLICATE SHAKING APPARATUS FOR DETERMINATION OF CARBONATE CARBON IN SOILS

This tube stands upright, extending thru a rubber stopper in a 1 liter Erlenmeyer flask, and to prevent the mechanical carrying over of any of the purifying NaOH it has inserted in the top a large N distillation bulb.

The driving wheel (*J*) is ½ inch thick and 7 inches in diameter. The eccentric attached to its face is ½ inch in thickness and 2 inches in diameter. This eccentric is grooved to permit free rotation of the driving shaft, which is fastened to the end of the holder (*H*) by means of a binding post. Power for agitation is supplied by the motor (*I*), a sewing machine, or small desk fan motor. If the motor available has no rheostat, its speed can be easily controlled by a battery of 4 lamps. The motor is hinged upright on the support so that the pulley will rest upon the edge of the driving wheel. To reduce noise the pulley of the motor is inserted into a rubber stopper. Or the driving wheel (*J*) may be made to carry a belt that is driven by the pulley of a small motor.

The absorption towers (*D*) are at least 25 inches high and 1 inch in diameter. They contain alternating pockets of solid glass rods and small glass beads resting upon an inverted test tube 2½ inches long. The rubber connection on the intake cock of the tower ($D$) is used to disconnect the glass tube that extends to the rubber connection on the safety bulb tube leading from flask ($C$).

## 6 DETERMINATION

### *Method I—Volumetric*[2]

Pulverize the sample to pass a 60-mesh sieve, so as to expose fully to the action of the liberating acid any calcite that may be included in quartz crystals. For soils low in carbonates use a 10-, 25-, or 50-g charge in the quadruplicate shaking device described under 5.

Introduce the charge into the 300 cc evolution flask (C, Figs. 1 and 2), aspirate 5 min. to free the apparatus of atmospheric $CO_2$; release the suction, and then introduce 10, 25, or 50 cc of 0.5 $N$ NaOH or KOH soln into the absorption tower. Apply a suction of 5 inches and then introduce 60 cc of HCl (1+9) containing 5% of $SnCl_2$ upon the soil contained in the Erlenmeyer flask, regulating the intake of air by means of a screw cock placed just beyond the absorption tower. Agitate and aspirate for 60 min. at the rate of 3–4 bubbles per second. Then release the suction and draw off the absorbent soln into a 500 cc flask, washing the tower with a succession of fillings of $CO_2$-free $H_2O$ to a volume of 450 cc. Add 10 cc of neutral aqueous soln of $BaCl_2$ (made by dissolving 250 g of $BaCl_2.2H_2O$ in 1 liter of $H_2O$), make to volume, agitate, and allow to stand 4 hours. Titrate the excess of hydroxide, using phenolphthalein indicator. With a small-bore buret permitting split-drop readings to hundredths of a cc use 0.5 $N$ acid for the titration; with burets of larger bore use 0.1 or 0.25 $N$ acid. Calculate and report the result as percentage of carbonate C or $CO_2$.

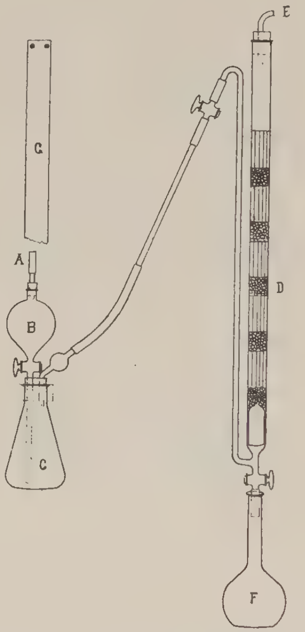

FIG. 2.—ABSORPTION TOWER

### 7 *Method II—Gravimetric*[12]

Proceed as in **6**, but in lieu of the NaOH and KOH absorbent use an absorption tube filled with ascarite. This tube should be preceded in the train by tubes containing an $Ag_2SO_4$ suspension in $H_2SO_4$(1+19), $H_2SO_4$, and $CaCl_2$ in order. Report the increase in the weight found for the ascarite tube in percentage of carbonate C or $CO_2$.

NOTE.—Special consideration should be given to the soils that have been treated with magnesite or dolmite or those known to be derived from the limited magnesite area, or from the glaciated region where transported dolomite may occur in considerable quantities. For such soils, agitate the HCl–$SnCl_2$-soil suspension until $CO_2$ evolution has subsided. Then apply heat to the agitated suspension until no $CO_2$ evolution is indicated by the liquids in the absorption train. Remove heat, discontinue agitation, and draw $CO_2$-free air thru the apparatus for 20 min. The absorption may be accomplished by either the volumetric (**6**) or the gravimetric (**7**) procedure.

### *Method III—Furnace Combustion Method*[3]

8 APPARATUS

(a) *Oxygen cylinder.*—With pressure-regulating valve.

(b) *Electric combustion furnace.*—With rheostat, and with $\frac{7}{8}$ x 24-inch fused silica tube containing an 8-inch loosely-packed core of platinized asbestos. (CuO may be used if a temp. of 950° is not exceeded.)

(c) *Purification and absorption train.*—Place 2 scrubber-bottles containing a 10% soln of KOH, followed by an Hg valve, between the O supply and the intake end of the furnace. Provide an asbestos-filled Cu coil with handle as an insulating plug on the intake end of the combustion tube; also use an asbestos plug to insulate the rubber stopper at the outlet end of the combustion tube. The outcoming current is dried and purified by an $H_2SO_4$ scrubber, a tube containing 40-mesh granulated Zn and a tube of $P_2O_5$, or equivalent drying material, in order. The drying tube is connected with a Nesbit absorption bulb filled with alternate layers of glass wool and ascarite. The Nesbit tube is protected against moisture and back pressure at its outlet by a Fisher bubble counter containing concentrated $H_2SO_4$.

9 DETERMINATION

Bring the furnace to a temp. of 900–950°. Connect the train and sweep out the apparatus by an adjusted flow of O. Weigh the Nesbit tube against a counterpoise. Replace the Nesbit tube in the train and introduce well within the heated zone an alundum boat containing a 2-g charge of soil admixed with 2 g of finely divided CuO. Close the intake and open the Nesbit bulb. When no further flow of gas passes thru the absorption train, connect with suction, admit a flow of O, and aspirate for 30 min. Close the Nesbit tube, disconnect, and weigh against the counterpoise. Correct the total evolution of $CO_2$ for the carbonate $CO_2$ (determined as **6** or **7**) and report as organic C, or $CO_2$. There should be no carbonates in the residue in the case of acid, calcareous or dolomitic soils. Unleached alkali soils should be corrected for the original and any residual $CO_2$.

## ORGANIC CARBON

### *Wet Oxidation Method*

10 REAGENTS

(a) *Oxidizing soln.*—Dissolve 85 g of $CrO_3$ in 100 cc of $H_2O$ and make to 250 cc with 85% $H_3PO_4$.

(b) *Acid soln.*—Mix equal volumes of 85% $H_3PO_4$ and boiled concentrated $H_2SO_4$.

11 DETERMINATION

Introduce 1–5 g of soil, depending upon the organic matter content, into each of the four 300 cc Pyrex Erlenmeyer flasks, **5**. In addition, insert a glass bulb of about $1\frac{1}{2}$ inches diameter between the Erlenmeyer flasks and the absorption towers and bend the tube leading from this bulb into the Erlenmeyer flask so as to permit the return of the condensed $H_2O$ along the side of the flask. In order to guard against the mechanical aspiration of the liberating acids, place an empty absorbent bead-filled tower between each glass bulb and the tower that contains the hydroxide absorbent. Free the apparatus of atmospheric $CO_2$ and then introduce into each absorption tower 25 or 50 cc of 0.5 $N$ NaOH or KOH. Apply suction of 5 inches and run into each Erlenmeyer flask 10 cc of the oxidizing soln. Then add 25–40 cc of the acid mixture. Gently agitate the flasks and place a low flame under each. Continue the gentle agitation and heating for 30 min. after the mixture begins to boil.

At the end of the agitation and aspiration, release the suction and wash the absorbent into a 500 cc flask. Precipitate the $Na_2CO_3$ or $K_2CO_3$ by adding 10 cc of a neutral aqueous soln of $BaCl_2$ (250 g of $BaCl_2.2H_2O$ per liter). Dilute to volume and permit the precipitate of $BaCO_3$ to settle 4 hours. Pipet an aliquot of 200 cc and titrate the residual hydroxide with 0.5 *N* acid, using phenolphthalein indicator and splitting drops by the use of a stirring rod near the end of the titration.

Residual hydroxide in terms of 0.5 *N* alkali minus the 0.5 *N* NaOH originally used = the $CO_2$ present in the sample. Total $CO_2$, as determined under **6** = percentage of carbonate C or $CO_2$ derived from the oxidation of the organic material.

### TOTAL NITROGEN

**12** *Gunning-Hibbard Method*

Digest 10 g of soil in a 500 cc Kjeldahl boiling flask with 30–40 cc of concentrated $H_2SO_4$ and approximately 10 g of salt mixture composed of 10 parts of $K_2SO_4$ or annhydrous $Na_2SO_4$, 1 part of $FeSO_4$, and $\frac{1}{2}$ part of $CuSO_4$. Continue the digestion until the mixture is colorless or nearly so. After cooling, dilute the contents of the flask with $H_2O$, add an excess of an approximately 45% NaOH soln, connect with the condenser, and distil 150 cc into standard acid as directed under **II, 19.** The distillation may be carried out in the digestion flask, or, if preferred, the soln may be transferred to a Cu flask. Titrate the excess of acid with 0.1 *N* or *N*/14 alkali, using methyl red or cochineal indicator. Report as percentage of N.

**13** *Kjeldahl Method*

Proceed as directed under **12,** using 0.7 g of HgO or 0.65 g of Hg instead of the salt mixture. Mix immediately and heat over a low flame, gradually increasing the heat. Continue the digestion until the mixture is colorless or nearly so. After cooling, dilute the contents of the flask; add 25 cc of sulfide or thiosulfate soln [**II, 17** (**h**)] and an excess of the NaOH soln; and proceed with the distillation and titration as directed under **II, 19.** Report as percentage of N.

### SODIUM CARBONATE FUSION[4]

**14** *Method I*

Thoroly mix on glazed paper 5 g of soil, ground to an impalpable powder, with 25 g of $Na_2CO_3$, and transfer carefully to a 100 cc Pt or Ni crucible. Cover, heat at low redness until fusion begins, then increase the heat until a clear, quiet fusion results; finally, give full heat of a Meker burner for 20 min., having the flame oblique to insure good oxidation. Pour the fusion into a large Pt dish set in water. Place the crucible and cover in a wide 400 cc beaker. Cover with water, transfer the fused lump from the Pt dish to the beaker, and rinse the dish into the beaker with HCl (1+9). Add 50 cc of HCl to the contents of the beaker, cover, and keep on a steam bath until the fused mass has disintegrated. Transfer the mixture to the Pt dish and evaporate to dryness on a steam bath.

**15** *Method II*

Proceed as directed under **27.**

**16** SILICA

Take up the residue from **14** or **15** in HCl (1+9) and filter the mixture so obtained (a 9 cm Büchner funnel with suction may be used advantageously). Wash with hot

$H_2O$ containing 5 cc of HCl per liter. Collect the filtrate and washings in a dish, preferably a casserole, and dehydrate on a steam bath until the $SiO_2$ assumes a crystalline appearance. Moisten with HCl and repeat the dehydration for 2 hours. Add 5 cc of HCl and 100 cc of hot $H_2O$. Mix thoroly, filter, and wash. Add the residue to the main portion of $SiO_2$ obtained from the first filtration. Make up the combined filtrate and washings to 500 cc at 20° and save for subsequent determinations. Place the two $SiO_2$ residues with filters in a porcelain crucible. Moisten with a saturated $NH_4NO_3$ soln. Ignite with low heat at first to burn off filter paper and then with a strong flame, preferably a blast lamp, to constant weight; cool in a desiccator; and weigh. Report as percentage of $SiO_2$.

### 17 OXIDES OF IRON, ALUMINUM, MANGANESE, PHOSPHORUS AND TITANIUM

To a 100 or 200 cc aliquot of the soln from **16**, according to the probable quantity of Fe and Ca present, add $NH_4OH$ (1+1) dropwise until the precipitate formed requires several seconds to dissolve, thus leaving the soln faintly acid. Add 0.5 g of solid $NH_4$-persulfate, heat nearly to the boiling point, and add sufficient $NH_4OH$ (1+1) to precipitate all Fe, Al, etc. Allow the mixture to boil in a covered beaker for about 1 min., remove, and if no $NH_3$ is given off (as detected by smelling), again add $NH_4OH$ dropwise until it can be detected. Do not allow the precipitate to settle, but stir and pour on the filter. Wash immediately with hot 2.5% $NH_4NO_3$ soln, playing a fine jet around the edge of the precipitate, thus cutting it free from the paper in order to insure rapid filtration. Wash the precipitate several times. Transfer the paper and the precipitate to the original beaker. Add 5 cc of HCl, macerate quickly, add 50 cc of $H_2O$, and heat. (This procedure insures that the paper will not be carried to the gel state.) Reprecipitate the oxides with $NH_3$-persulfate and dilute $NH_4OH$ as directed above, filter, and wash until free from chlorides with hot 2.5% $NH_4NO_3$ soln. Reserve the filtrate and washings from both the first and second precipitations for the determination of Ca and Mg.

Dry the precipitate, remove from the filter, and ignite in a Pt crucible over a Bunsen flame, incinerating the filter separately, and add the residue to the precipitate. Then ignite to bright redness, cool in a desiccator, and weigh as $Fe_2O_3$, $Al_2O_3$, $Mn_3O_4$, $TiO_2$, and $P_2O_5$.

To this residue add $KHSO_4$, or $K_2S_3O_7$, and heat at low temp. until the precipitate is completely disintegrated; cool quickly and transfer to a flask containing 100 cc of $H_2SO_4$ (1+3). Dissolve the melt, reduce with Zn, cool, and determine Fe by titration with 0.2 *N* $KMnO_4$ soln. (cf. **XXXVII, 58.**). Report as percentage of $Fe_2O_3$.

Or, in lieu of the above fusion, evaporate 50 or 100 cc of the soln from **16** after the addition of 10 cc of $H_2SO_4$ until all HCl is expelled. Dilute with $H_2O$, reduce with Zn, and determine the Fe by titration with 0.2 *N* $KMnO_4$ soln.

Evaporate 50–100 cc of the soln from **16** after the addition of 10 cc of $HNO_3$. Repeat the addition of $HNO_3$ and evaporation to insure expulsion of all HCl. From this point proceed as directed under **XXXVII, 75.** Subtract the sum of the oxides of Fe, Mn, and P (determined separately as directed in **29** or **30**) from the weight of the combined oxides of Fe, Al, Mn, P, and Ti, determined as directed above. Report the remainder as oxides of Al and Ti.

### 18 CALCIUM

Concentrate the combined filtrates and washings from **17** to about 50 cc; make slightly alkaline with $NH_4OH$ (1+1); and add, while still hot, saturated $NH_4$-oxalate soln dropwise as long as any precipitate is produced, and then an excess sufficient to convert the Mg salts also into oxalate. Heat to boiling, allow to stand 3 hours or longer, decant the clear soln thru a filter, pour 15–20 cc of hot $H_2O$ on the precipitate, and again decant the clear soln thru the filter. Dissolve the precipitate in the beaker with a few drops of HCl; add a little $H_2O$; and reprecipitate, boiling hot, by adding $NH_4OH$ and a little $NH_4$-oxalate soln. Allow to stand as before and filter thru the same filter. Wash free from chlorides with hot $H_2O$. Reserve the filtrates and washings from both precipitations for the determination of Mg under **19** or **21**. Complete the determination by one of the following procedures and report as percentage of CaO.

(**a**) Ignite the precipitate in a crucible over a S-free blast to constant weight, cool in a desiccator, and weigh the CaO.

(**b**) Incinerate the filter over a low flame, mix the ignited precipitate with a finely pulverized and dried mixture of equal parts of $(NH_4)_2SO_4$ and $NH_4Cl$, and drive off the excess of the sulfate by careful heating of the upper portion of the crucible. Complete the ignition, cool in a desiccator, and weigh the $CaSO_4$.[5]

(**c**) Dissolve the Ca-oxalate precipitate from the filter with hot $H_2SO_4$ (1+1), collect the soln in the beaker employed for precipitation, and titrate while hot with a standard soln of $KMnO_4$ (cf. **XII, 9**). Finally add the filter paper unmacerated to the soln and complete the titration.

## MAGNESIUM

**19**

### *Method I*

Evaporate the combined filtrates and washings from **18** on a water bath to about 100 cc and add cautiously 20 cc of $HNO_3$. Evaporate to dryness and heat carefully on a hot plate to remove $NH_4$-salts. Add 5 cc of HCl and evaporate nearly to dryness. Dissolve the residue in hot $H_2O$ and a small quantity of HCl. If necessary, filter the soln and wash the filter paper with about 100 cc of hot $H_2O$. Precipitate the Mg as $MgNH_4PO_4$ by the addition of 3 cc of a 10% soln of $NH_4$ phosphate and sufficient $NH_4OH$ to make the soln slightly alkaline. Stir the soln vigorously, allow to stand 15 min., add 15 cc of $NH_4OH$, and allow the precipitation to proceed overnight. Filter, wash the precipitate with $NH_4OH$ (1+9), transfer to a porcelain crucible, ignite, cool, and weigh as $Mg_2P_2O_7$. The filtration may be made thru a Gooch crucible. Calculate and report the result as percentage of MgO.

### *Method II*[6]

**20**

#### REAGENTS

(**a**) *Sodium ammonium phosphate soln.*—Dissolve 100 g of $NaNH_4HPO_4.4H_2O$ in hot $H_2O$, cool, and dilute to 1 liter.

(**b**) *Dilute ammonium hydroxide for washing.*—Dilute 100 cc of $NH_4OH$ to 1 liter.

**21**

#### DETERMINATION

To the combined filtrates and washings from **18**, add 100 cc of $NH_4OH$ and 50 cc of 95% alcohol. Then add with constant stirring 25 cc of the $NaNH_4$-$HPO_4$ soln and let stand 12–24 hours. Filter, wash twice with the dilute $NH_4OH$, and dissolve the precipitate in $HNO_3$ (1+4), washing the soln into the original beaker to a volume of 100–150 cc. To this soln add $\frac{1}{3}$ volume of $NH_4OH$, 25 cc of 95% alcohol, and 2 drops of the Na-$NH_4$-phosphate soln. Stir vigorously and allow to stand 3 hours or longer. Filter thru a Gooch crucible, wash with the $NH_4OH$, ignite, and weigh as $Mg_2P_2O_7$. Calculate and report the result as percentage of MgO.

## MANGANESE

### *Method I*

**22**

#### REAGENTS

The reagents and solns used are described under **XXXVII, 74.**

### 23 DETERMINATION

Treat 1 g of 100-mesh soil with 5 cc of HF and 5 cc of $H_2SO_4(1+1)$. Evaporate to dryness, ignite, and fuse the residue with $KHSO_4$. Repeat the addition of HF until all silicates are decomposed. Dissolve in $H_2O$, add $HNO_3$, and evaporate to dryness. Again dissolve in $H_2O$; add 25 cc of $HNO_3$ $(1+2)$, then add about 0.5 g of Na-bismuthate; and heat until the permanganate color disappears. From this point proceed as directed under **XXXVII, 75**, beginning with "Add a few drops of a soln of $NH_4$- or Na-bisulfite to clear the soln." Report as percentage of mangano-manganic oxide ($Mn_3O_4$).

## *Method II*[7]

### 24 REAGENTS

(**a**) *Sulfuric acid soln.*—$(1+1)$.

(**b**) *Potassium bisulfate.*—Mn-free and finely pulverized.

(**c**) *Standard manganous sulfate soln.*—Dissolve 0.2877 g of pure $KMnO_4$ in about 100 cc of $H_2O$, acidify the soln with $H_2SO_4$ $(1+1)$, and slowly heat to boiling. Add slowly a sufficient quantity of a 10% oxalic acid soln to discharge the color. Cool, and dilute to 1 liter. 1 cc of this soln $=0.1$ mg of Mn.

(**d**) *Potassium periodate.*

### 25 DETERMINATION

Weigh 0.5–5.0 g of finely pulverized, air-dried soil into a 50 cc $SiO_2$ or Pt crucible. Add to the soil approximately $2\frac{1}{2}$ times its weight of finely powdered, Mn-free $KHSO_4$ and mix thoroly. Place the lid on the crucible and heat gently over a Bunsen burner about 5 min.; increase the heat gradually until the crucible and lid are red hot, being careful not to allow the contents of the crucible to froth over. Continue to heat for about 20 min., or until the frothing has ceased and the contents are in a quiet molten condition. Withdraw the flame from beneath the crucible, remove the lid, and rotate the crucible in a horizontal position to spread the molten contents over the inner walls to expedite cooling. When the crucible is no longer red, immerse it in about 25 cc of $H_2SO_4$ $(1+1)$ in a 250 cc beaker and digest on a hot water bath until the contents of the crucible disintegrate and dissolve. Carefully rinse the crucible and lid with hot $H_2O$ and dilute the soln to about 100 cc. Filter, and wash the insoluble residue.

Discard the insoluble residue if it has a uniform white color; if it is colored by undecomposed particles of minerals, ignite and expel the $SiO_2$ with HF and $H_2SO_4$. Fuse the residue with $KHSO_4$, digest in dilute $H_2SO_4$, and add the soln to the filtrate from the fusion.

Make the soln to a definite volume, take an aliquot for the determination, and add about 0.05 g of K-periodate to the aliquot. Boil the soln until the characteristic purplish permanganic acid color develops, heat on a hot water bath for an hour, and set aside to cool. If the color is deep purple, dilute the soln to a definite volume. Remove an aliquot and match against a standard Mn soln in Nessler jars, or in a colorimeter. Compute the results as percentage of Mn or $Mn_3O_4$. (A series of Mn standard solns are prepared from reagent (**c**) by removing aliquots of the $MnSO_4$ soln and developing the Mn color with K-periodate in the same way as the soln of the sample.

### 26 IODINE[8]

Weigh 50 g of air-dried soil into a quartz boat and introduce into the quartz combustion tube of an electric furnace. Attach 3 absorption bottles containing thin suspensions of $Ca(OH)_2$ and connect with suction apparatus. To the other end of the furnaces attach 2 bottles containing 5% KOH soln. Heat the furnace to a temp. of 1100° and aspirate with I-free air for 2 hours. Cool, wash the contents of the $Ca(OH)_2$ absorption train into a large beaker and digest for 30 min. Filter, and wash thoroly. Transfer the filtrate to a porcelain dish and evaporate to dryness. Moisten the residue with a few cc of hot $H_2O$, transfer to a small quartz dish, add 2 cc of a $Ca(OH)_2$ suspension, evaporate to dryness, and ignite gently to remove traces of organic matter. Cool, moisten the ignited residue with hot $H_2O$, and filter to a volume of 10 cc in a 30 cc separatory funnel. Add 1 cc each of $CS_2$, $H_2SO_4$ (1+9) and 1% $NaNO_2$; stopper tightly and shake vigorously for 1 min. Test an aliquot of a standard KI soln similarly in a second funnel. Compare the pink colors and compute the values in terms of parts per billion of soil.

## SULFUR[9]

### 27 PREPARATION OF SOLUTION

Weigh 5–10 g of the soil, prepared as directed under **2(b)** to pass a 0.5 mm sieve, into a 100 cc Ni crucible; add an equal weight of anhydrous $Na_2CO_3$; and mix well with a stout Ni stirring rod of such length as to permit introduction into the furnace to be used in the fusion. Pipet carefully 4 cc of $H_2O$ into each 10 g of soil; stir well to a stiff paste, adding more $H_2O$ if necessary, a few drops at a time. Immediately add successive portions of about 1 g of S-free $Na_2O_2$, stirring well after each addition to obviate excessive frothing and overflow. Continue to add peroxide until the mixture becomes dry and granular, and then add, as a surface coating, enough to make the total peroxide addition 25 g per each 10 of soil. Place the mixture in an electric furnace; maintain a temp. between 400° and 500° during the first half hour, then raise it rapidly to bright red heat (about 900°); and continue the fusion at this temp. for about 10 min. Withdraw the crucible from the muffle, quickly manipulate so as to cause the melt to spread out in a thin sheet over the interior of the crucible, and cool rapidly by contact with some good conductor in a cool atmosphere. Place the chilled crucible sideways in a 600 cc beaker and cover with $H_2O$. Add about 5 cc of 95% alcohol to decompose the Na-manganate. Cover the beaker with a watchglass, place on a cold hot plate, and apply heat. Boil briskly until all the melt is disintegrated (30 min. is ordinarily sufficient). When the suspension has assumed a flesh-colored, flocculent appearance, with no glassy green lumps in the interior of the crucible, remove the crucible and rod from the beaker and wash any flaky particles back into the beaker with the aid of a rubber-tipped glass rod, rinsing several times with hot $H_2O$. (If small glassy particles still cling to the inside of the crucible, disintegrate by boiling $H_2O$ over the hot plate or a small flame and add to the main portion.) Filter immediately by suction thru a 9 cm Büchner funnel into a liter beaker placed under a bell jar. When no more of the liquid can be drawn thru the filter, return the residue, together with the filter paper, to the original beaker, washing any adhering particles carefully from the funnel. Add about 1 g of $Na_2CO_3$, macerate with the stirring rod, add 75–100 cc of $H_2O$, and bring to a brisk boil while stirring vigorously. Again filter thru a Büchner funnel, using suction, until nearly dry, and wash with 20 cc portions of hot $H_2O$ to a total volume of 500 or 700 cc for the 5 and 10 g charges, respectively.

### 28 DETERMINATION

Add from a buret slowly and with stirring, sufficient HCl to neutralize the soln, using methyl red indicator [**II, 17(k)**]. Add 0.5 cc excess of the HCl and concentrate by heating to a volume of 400 cc. Heat to boiling, add slowly 10 cc of a 5%

$BaCl_2$ soln, and allow to stand overnight. Filter on a dense filter paper, place paper in a Pt crucible, and ignite in an electric furnace. Cool in a desiccator, and weigh as $BaSO_4$. To insure against possible inclusion of $SiO_2$, add 2 drops of HF and 1 drop of $H_2SO_4$ (1+1), heat carefully, reignite, and weigh. Report as percentage of S or $SO_3$.

## PHOSPHORUS

### 29 *Sodium Peroxide Method*

Place 10 g of $Na_2O_2$ in an iron or porcelain crucible and thoroly mix with it 5 g of the soil prepared as directed under **2(b)**. If the soil has very little organic matter, add a little starch to hasten the action. Heat the mixture carefully by applying the flame of a Bunsen burner directly upon the surface of the charge and the sides of the crucible until the action starts. Cover the crucible until reaction is over and keep at a low red heat for 30 min. Do not allow fusion to take place. By means of a large funnel and a stream of hot $H_2O$ transfer the charge to a 500 cc volumetric flask, acidify with HCl and boil. Cool, and make up to the mark. (If the action has taken place properly, there should be no particles of undecomposed soil in the bottom of the flask.) Allow the $SiO_2$ to settle and draw off 200 cc of the clear soln.

Or, in lieu of the above, oxidize 5–10 g of the material and disintegrate the melt as directed under 27. Acidify with HCl, dilute to 500 cc, and withdraw a 200 cc aliquot.

Precipitate the Fe, Al, and P with $NH_4OH$ (1+1); filter; wash several times with hot $H_2O$; return the precipitate to the beaker; and dissolve it in hot HCl (1+4), pouring the acid upon the filter to dissolve any precipitate remaining. Evaporate the soln and washings to complete dryness on a water bath. Take up with $HNO_3$ (1+4), heating if necessary, and filter to remove $SiO_2$. Evaporate the filtrate and washings to about 10 cc and add 2 cc of $HNO_3$. Neutralize the excess of acid with $NH_4OH$ (1+1) and then add $HNO_3$ until the soln becomes clear, avoiding an excess of the acid. Heat to 40–50° in a water bath, add 15 cc of molybdate soln [**II, 5(a)**] and keep at this temp. for 1–2 hours. Let stand overnight, filter, and wash free from acid with cold $H_2O$. Transfer the filter to a beaker and dissolve in standard NaOH or KOH (1 cc = 0.5 mg of $P_2O_5$). Titrate the excess of alkali with standard $HNO_3$, using phenolphthalein indicator. Or, after adding the 15 cc of molybdate soln, allow to stand 3 hours at a temp. not above 60°, filter on a small filter or on a Gooch crucible, and wash with cold $H_2O$ until two fillings of the filter do not greatly diminish the color produced with phenolphthalein by 1 drop of standard alkali. Return the filter and precipitate to the same beaker used for precipitating the phosphomolybdate, dissolve the yellow precipitate in standard NaOH or KOH (1 cc = 0.5 mg of $P_2O_5$), add a few drops of phenolphthalein indicator, and titrate the excess of alkali with standard $HNO_3$. Report as percentage of $P_2O_5$.

### 30 *Magnesium Nitrate Method*

Place 5 g of soil, prepared as directed under **2(b)**, in a porcelain dish. Moisten with 5–7 cc of $Mg(NO_3)_2$ soln [**II, 5(e)**]. Dry on a water bath and burn off the organic matter at low redness. Cool; and add 10 cc of $H_2O$, 10 cc of HCl, and 5 cc of $HNO_3$. Cover the dish and digest the contents for 2 hours on a water bath, stirring 2 or 3 times during the digestion. Dilute to 250 cc, mix well, and filter thru a dry folded filter, pouring back thru the filter until the filtrate is clear. Place an aliquot corresponding to 2 or 4 g of the soil, depending upon the quantity of P present, in a hard glass beaker or porcelain dish and evaporate to dryness on a water bath. Take up with $HNO_3$ (1+4), again evaporate to dryness, and heat for 1 hour at 110–120°.

Again take up with the dilute $HNO_3$ and filter. Reduce the combined volume of filtrates and washings to 30-40 cc. Make alkaline with $NH_4OH$ (1+1), and dissolve the precipitate with $HNO_3$, using a slight excess. Add gradually with vigorous agitation 15 cc of molybdate soln [(**II, 5(a)**]. Keep the soln at 40–50° for an hour and then let stand overnight at room temp. Filter, and wash well with cold $H_2O$. Return filter and precipitate to the same flask and determine P volumetrically as directed under **29**. Report as percentage of $P_2O_5$.

### 31 POTASSIUM AND SODIUM,[10] OR POTASSIUM ONLY

Triturate gently 0.5 or 1 g of the finely ground soil with 1 g of dry $NH_4Cl$ in a smooth mortar, add 8 parts of $CaCO_3$, and mix intimately. Transfer the mixture to a Pt crucible, rinsing the mortar with a little $CaCO_3$. Heat the crucible gradually until fumes of $NH_4$-salts no longer appear and the lower $\frac{3}{4}$ of the crucible is brought to a red heat. Maintain this temp. 40–60 min. The temp. should be sufficient to keep the $CaCl_2$, formed by the reaction of $NH_4Cl$ with $CaCO_3$, in a state of fusion. (The mass does not become liquid because the fused $CaCl_2$ is absorbed by the large quantitiy of $CaCO_3$ present. If the silicate is fused by the application of too strong heat, disintegration of the mass at the end of the operation with $H_2O$ cannot be effected. Moreover, too high a temp. causes volatilization of alkali chlorides. The mass contracts in volume during the ignition and is usually easily detached from the crucible.) Transfer the fused mass to a porcelain dish, slake with hot $H_2O$, and grind thoroly with an agate pestle. After washing 5 times by decantation with hot $H_2O$, transfer to a filter and wash well (300 cc of wash water is sufficient). To the filtrate add sufficient $(NH_4)_2CO_3$ soln to precipitate the Ca and any Mg present. Allow to settle, decant the supernatant liquid into a porcelain dish, and concentrate by evaporation, finally transferring the precipitate to the dish. When the volume is reduced to about 30 cc add a little $(NH_4)_2CO_3$ and $NH_4OH$; heat; filter into a porcelain dish; evaporate the filtrate to dryness on a water bath; and expel $NH_4$-salts by ignition; or evaporate with 10 cc of $HNO_3$, followed by 2 evaporations with 10 cc of HCl.

If K alone is to be determined proceed from this point as directed under **II, 43(a)**, beginning with "Dissolve the residue in hot $H_2O$." Report as percentage of $K_2O$.

If both K and Na are to be determined, dissolve the residual alkali chlorides in 3–5 cc of $H_2O$; a little black or brown flocculent matter usually remains undissolved, and the soln may also contain traces of Ca. Add 2–3 drops of the $(NH_4)_2CO_3$ soln and $NH_4OH$, warm and filter thru a small filter into a weighed Pt dish. Evaporate to dryness on a water bath, carefully heat the residual alkali chlorides to incipient fusion, cool, and weigh as Na- and K-chlorides. Dissolve the combined chlorides in 30 cc of $H_2O$, add 1.5 cc of $PtCl_4$ soln [**II, 41(b)**], evaporate to a sirupy consistency, and add 15 cc of 2.25 *N* acidulated alcohol (prepared by passing HCl gas into a mixture of 2000 cc of 95% alcohol and 152 cc of HCl). Filter thru an asbestos Gooch and wash with the acidulated alcohol and then with 80% alcohol. Dry the Gooch for one hour in an electric oven and then weigh. Dissolve the potassium platinic chloride with hot $H_2O$; wash with 80% alcohol and again place in a drying oven for an hour. Cool in a desiccator, weigh, and calculate to $K_2O$. Calculate to KCl and deduct from the weight of combined Na- and K-chlorides to obtain NaCl. Calculate and report the result as percentage of $Na_2O$.

### 32 QUALITATIVE TEST FOR SOIL REACTION

Place strips of neutral litmus paper in the bottom of a number of Petri dishes; over these lay 1 or 2 thicknesses of filter paper (free from acid); and place the soil, prepared as directed under **2(a)**, on the filter paper. With a horn spoon or clean spatula press the soil down firmly against the paper and add enough $H_2O$ (tested and found neutral) to saturate the soil. Cover the dishes, allow to stand for 30 min., and then note the color of the test paper. A check Petri dish containing neutral litmus paper and filter paper, moistened with the same $H_2O$, is allowed to stand under the same conditions. The filter paper gives a uniform background and evenness of contact.

## REACTION VALUES

### 33 DETERMINATION[11]

Use either the colorimetric or electrometric method, as found convenient. Determine the reaction values on fresh moist samples, using a soil-to-water ratio of 1:5, with intermittent agitation for 30 min.

### METHOD OF STATEMENT

For simplicity and ease of interpretation use a dual system of statement, giving both pH values and their equivalents in arithmetically related numbers in parentheses, as tabulated below.

| SØRENSEN OR pH VALUES | SPECIFIC ACIDITY OR HYDROGEN-ION CONCENTRATIONS | SØRENSEN OF pH VALUES | SPECIFIC ALKALINITY OR HYDROXYL-ION CONCENTRATIONS |
|---|---|---|---|
| 7.0 | 0.0 | 7.0 | 0.0 |
| 6.9 | 0.5 | 7.1 | 0.5 |
| 6.8 | 1.0 | 7.2 | 1.0 |
| 6.7 | 1.5 | 7.3 | 1.5 |
| 6.6 | 2 | 7.4 | 2 |
| 6.5 | 3 | 7.5 | 3 |
| 6.4 | 4 | 7.6 | 4 |
| 6.3 | 5 | 7.7 | 5 |
| 6.2 | 6 | 7.8 | 6 |
| 6.1 | 8 | 7.9 | 8 |
| 6.0 | 10 | 8.0 | 10 |
| 5.9 | 12.5 | 8.1 | 12.5 |
| 5.8 | 16 | 8.2 | 16 |
| 5.7 | 20 | 8.3 | 20 |
| 5.6 | 25 | 8.4 | 25 |
| 5.5 | 31.5 | 8.5 | 31.5 |
| 5.4 | 40 | 8.6 | 40 |
| 5.3 | 50 | 8.7 | 50 |
| 5.2 | 63 | 8.8 | 63 |
| 5.1 | 80 | 8.9 | 80 |
| 5.0 | 100 | 9.0 | 100 |
| 4.9 | 125 | 9.1 | 125 |
| 4.8 | 160 | 9.2 | 160 |
| 4.7 | 200 | 9.3 | 200 |
| 4.6 | 250 | 9.4 | 250 |
| 4.5 | 315 | 9.5 | 315 |

### 34 NITRATE NITROGEN

Place 100 g of the air-dried soil, prepared as directed under **2(a)**, and 500 cc of $H_2O$ in a suitable container, and agitate for 5 min. Add 1 g of CaO or 2 g of precipitated $CaCO_3$, agitate thoroly, allow to stand 10–20 min., and filter, making sure that a clear filtrate is obtained. If the filtrate contains 6 parts of Cl per million or less, proceed as directed under **XXXVII, 17,** using 25 cc of the filtrate; if it contains more than 6 parts of Cl per million, proceed as directed under **XXXVII, 19,** using 25 cc or a quantity that will contain not to exceed 0.1 mg of N in the form of nitrate. Report as percentage of nitrate N.

## 35 ALKALI SALTS

To 100 g of soil in a 500 cc bottle, add 250 cc of $H_2O$. Stopper, shake thoroly, and allow to stand overnight. Filter thru a Pasteur-Chamberland filter. Evaporate 50 cc of the filtrate to dryness in a Pt dish on a steam bath, ignite at a low red heat to drive off organic matter, cool in a desiccator, and weigh for total salts. Dissolve the residue in the Pt dish in 10–15 cc of hot $H_2O$, transfer to a 50 cc volumetric flask, cool, and dilute to the mark.

For determination of Cl, titrate an aliquot of 10 cc against 0.1 $N$ $AgNO_3$ soln. Calculate and report the result as percentage of NaCl.

For determination of alkali carbonate, titrate an aliquot of 10 cc against 0.1 $N$ HCl. Calculate and report the result as percentage of $Na_2CO_3$.

Determine sulfates by difference. If much gypsum is present, filter the soln of the salts in hot $H_2O$ thru a small filter, weigh the gypsum separately, and subtract the amount from the total amount of sulfates.

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[11] J. Wash. Acad. Sci., **9,** 305 (1919); Ibid., **11,** 197 (1921); Ecology, **3,** 346 (1922); Am. J. Pharm., **2,** 59 (1927); J. Assoc. Official Agr. Chem., **12,** 64 (1929).
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# II. FERTILIZERS

## GENERAL METHODS

### 1 DIRECTIONS FOR SAMPLING[1]—OFFICIAL

Each official sample sent to the laboratory shall consist of at least 1 pound of the material taken in the following manner: Use a sampler that removes a core from the top to the bottom of the bag. Take cores from not less than 10 per cent of the bags present unless this process necessitates cores from more than 20 bags, in which case take a core from 1 bag for each additional ton represented. If less than 100 bags, sample not less than 10 bags; if less than 10 bags, sample all bags. Thoroly mix the portions taken on a clean oilcloth or paper, reduce by quartering to the quantity of sample required, and place in an air-tight container.

### 2 PREPARATION OF SAMPLE—OFFICIAL

Pass the entire sample submitted to the chemist thru a 10-mesh sieve before subdividing for analysis. Reduce the gross sample by quartering to a quantity sufficient for analytical purposes. Transfer the sample to a sieve having circular openings 1/25 inch (1 mm) in diameter and sift, breaking the lumps with a pestle. Grind the portion remaining on the sieve until all particles pass thru, grinding and sifting as rapidly as possible to avoid loss or gain of moisture during the operation. Mix thoroly and preserve in tightly stoppered bottles.

### 3 MECHANICAL ANALYSIS OF BONE AND TANKAGE—OFFICIAL

Transfer 100 g of the original material to a sieve having circular openings 1/50 inch (0.5 mm) in diameter. Sift, breaking the lumps by means of a soft rubber pestle if the material has a tendency to cake. Weigh the coarse portion remaining on the sieve. Determine the fine portion by difference.

### 4 MOISTURE—OFFICIAL

Heat 2 g of the sample prepared as directed under **2** for 5 hours in a water oven at the temperature of boiling $H_2O$ (98–100°). In the case of potash salts, $NaNO_3$, and $(NH_4)_2SO_4$, heat at about 130° to constant weight. Report the percentage loss in weight as moisture.

### TOTAL PHOSPHORIC ACID

#### *Gravimetric Method—Official*

#### 5 REAGENTS

(a) *Molybdate soln.*—Dissolve 100 g of molybdic acid ($MoO_3$) in a mixture of 144 cc of $NH_4OH$ and 271 cc of $H_2O$. Pour this soln slowly and with constant stirring into a mixture of 489 cc of $HNO_3$ and 1148 cc of $H_2O$. Keep the final mixture in a warm place for several days or until a portion heated to 40° deposits no yellow precipitate of $NH_4$-phosphomolybdate. Decant the soln from any sediment and preserve in glass-stoppered vessels.

(b) *Ammonium nitrate soln.*—Dissolve 100 g of commercial $NH_4NO_3$, phosphate-free, in $H_2O$ and dilute to 1 liter.

(c) *Magnesia mixture.*—(1) Dissolve 11 g of MgO in HCl (1+4), avoiding an excess of the acid; add a little MgO in excess; boil a few minutes to precipitate Fe, Al, and $P_2O_5$; and filter. To the filtrate add 140 g of $NH_4Cl$ and 130.5 cc of $NH_4OH$

and dilute to 1 liter. Or, (2) dissolve 55 g of crystallized $MgCl_2.6H_2O$ in $H_2O$, add 140 g of $NH_4Cl$ and 130.5 cc of $NH_4OH$, and dilute to 1 liter. Or, (3) dissolve 55 g of crystallized $MgCl_2.6H_2O$ in $H_2O$, add 140 g of $NH_4Cl$, and dilute to 870 cc. Add $NH_4OH$ to each required portion of the soln just before using, at the rate of 15 cc per 100 cc of soln.

(d) *Ammonium hydroxide for washing.*—Dilute 100 cc of $NH_4OH$ to 1 liter. (This soln should contain not less than 2.5% of $NH_3$ by weight.)

(e) *Magnesium nitrate soln.*—Dissolve 150 g of MgO in $HNO_3$ (1+1), avoiding an excess of the acid; add a little MgO in excess; boil; filter from the excess of MgO, $Fe_2O_3$, etc.; and dilute to 1 liter.

### 6 PREPARATION OF SOLUTION

Treat 2 g of the sample by one of the methods given below. Cool the soln, dilute to 200 cc, mix, and pour on a dry filter.

(a) Dissolve in 30 cc of $HNO_3$ and 3–5 cc of HCl and boil until the organic matter is destroyed. (Suitable for materials containing small quantity of organic matter.)

(b) Dissolve in 15–30 cc of HCl and 3–10 cc. of $HNO_3$. (This method is recommended for fertilizers containing much Fe- or Al-phosphate).

(c) Evaporate with 5 cc of $MgNO_3$, ignite, and dissolve in HCl. (Suitable for organic material like cottonseed meal alone or in mixtures.)

(d) Boil with 20–30 cc of $H_2SO_4$ in a 200 cc flask, adding 2–4 g of $NaNO_3$ or $KNO_3$ at the beginning of the digestion and a small quantity after the soln has become nearly colorless, or adding the nitrate in small portions from time to time. When the soln is colorless, add 150 cc of $H_2O$ and boil for a few minutes. (Generally applicable to materials or mixtures containing large quantities of organic matter. With cottonseed meals and materials of like nature it is best to add first about 5 cc of $HNO_3$ and then the $H_2SO_4$. Allow the mixture to digest, at a gentle heat if necessary, until the violence of the reaction is over before adding the nitrate.)

### 7 DETERMINATION

Pipet an aliquot of the soln (prepared as directed under **6**) corresponding to 0.25 g, 0.50 g, or 1 g, into a 250 cc beaker; add $NH_4OH$ in slight excess; and barely dissolve the precipitate formed with a few drops of $HNO_3$, stirring vigorously. If HCl or $H_2SO_4$ has been used as a solvent, add about 15 g of crystalline $NH_4NO_3$ or a soln containing that quantity. To the hot soln add 70 cc of the molybdate soln for every decigram of $P_2O_5$ present. Digest at about 65° for 1 hour, and determine whether or not the $P_2O_5$ has been completely precipitated by the addition of more molybdate soln to the clear supernatant liquid. Filter, and wash with cold $H_2O$ or preferably with the $NH_4NO_3$ soln. Dissolve the precipitate on the filter with $NH_4OH$ (1+1) and hot $H_2O$ and wash into a beaker to a volume of not more than 100 cc. Neutralize with HCl, using litmus paper or bromthymol blue as an indicator; cool; and from a buret add slowly (about 1 drop per second), stirring vigorously, 15 cc of the magnesia mixture for each decigram of $P_2O_5$ present. After 15 min. add 12 cc of $NH_4OH$. Let stand until the supernatant liquid is clear (usually 2 hours), filter, wash the precipitate with the dilute $NH_4OH$ until the washings are practically free from chlorides, dry, burn first at a low heat and ignite to constant weight, preferably in an electric furnace, at 950–1,000°; cool in a desiccator, and weigh as $Mg_2P_2O_7$. Calculate and report the result as percentage of $P_2O_5$.

### *Volumetric Method—Official*

### 8 REAGENTS

(a) *Molybdate soln.*—To 100 cc of molybdate soln, prepared as directed under **5**(a), add 5 cc of $HNO_3$. Filter this soln immediately before using.

(b) *Standard sodium or potassium hydroxide soln.*—Dilute 323.81 cc of *N* alkali, free from carbonates, to 1 liter; 100 cc of the soln should neutralize 32.38 cc of *N* acid; 1 cc = 1 mg or 1% of $P_2O_5$ on a basis of 0.1 g of substance.

(c) *Standard acid soln.*—Prepare a soln of HCl or of $HNO_3$ corresponding to the strength of (b), or to ½ of this strength, and standardize by titration against that soln, using the phenolphthalein indicator.

(d) *Phenolphthalein indicator.*—Dissolve 1 g of phenolphthalein in 100 cc of alcohol, 95% by volume.

### 9 PREPARATION OF SOLUTION

Treat 2 g of the sample as directed under **6**(a), (b), (c) or (d), preferably (a) when these acids are a suitable solvent, and dilute to 200 cc with $H_2O$.

### 10 DETERMINATION[6]

(a) For percentages up to 5 use an aliquot corresponding to 0.4 g of substance; for percentages between 5 and 20 use an aliquot corresponding to 0.2 g of substance; and for percentages above 20 use an aliquot corresponding to 0.1 g of substance. Add 5-10 cc of $HNO_3$, depending on the method of soln (or the equivalent in $NH_4NO_3$); add $NH_4OH$ until the precipitate that forms dissolves but slowly on stirring vigorously; dilute to 75–100 cc; and adjust to a temp. of 25-30°. For percentages below 5, add 20-25 cc of the freshly filtered molybdate soln; for percentages between 5 and 20 add 30–35 cc of the molybdate soln; and for percentages greater than 20 add sufficient molybdate soln to insure complete precipitation. Place the soln in a shaking or stirring apparatus and shake or stir for 30 min. at room temp.; decant *at once* thru a filter; and wash the precipitate twice by decantation with 25–30 cc portions of $H_2O$, agitating thoroly and allowing to settle; then transfer the precipitate to the filter and wash with cold $H_2O$ until the filtrate from 2 fillings of the filter yields a pink color upon the addition of phenolphthalein and 1 drop of the standard alkali. Transfer the precipitate and filter to the beaker or precipitating vessel, dissolve the precipitate in a small excess of the standard alkali, add a few drops of phenolphthalein indicator, and titrate with the standard acid.

(b) *Not applicable in the presence of sulfates.*—Proceed as directed under (a) to the point where the soln is diluted to 75–100 cc. Then heat in a water bath to 45–50°, add the molybdate soln at the rate of 75 cc for each decigram of $P_2O_5$ present; allow the mixture to remain in the bath, stirring occasionally for 30 min.; decant *at once* thru a filter, wash, and titrate as directed under (a).

## WATER-SOLUBLE PHOSPHORIC ACID

### 11 *Gravimetric Method—Official*

Place 2 g of the sample* on a 9 cm filter and wash with successive small portions of water, allowing each portion to pass thru before adding more, until the filtrate measures about 250 cc. If the filtrate is turbid, add 1–2 cc of $HNO_3$. Dilute to a convenient volume, mix well, and proceed as directed under **7**.

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* The use of 1 g (instead of 2 g as given here) followed by 1 hour's digestion (see sec. **14**) was approved as official, first action, at the 1930 meeting, but this change will not become official until final action has been taken.

### 12 *Volumetric Method—Official*

Treat the sample as directed under **11.** To an aliquot of the soln corresponding to 0.2 or 0.4 g, add 10 cc of $HNO_3$, nearly neutralize with $NH_4OH$, dilute to 60 cc, and proceed as directed under **10.**

## CITRATE-INSOLUBLE PHOSPHORIC ACID—OFFICIAL

### 13 REAGENTS

*Ammonium citrate soln.*—Prepare according to either of the following methods, preferably (2):

(1) Dissolve 370 g of crystallized citric acid in 1500 cc of $H_2O$, nearly neutralize with strong $NH_4OH$, cool, add strong $NH_4OH$ until exactly neutral to corallin (saturated alcoholic soln), and dilute sufficiently to make the sp. gr. 1.09 at 20°. The volume will be about 2 liters.

(2)[2] For every liter of soln required dissolve 172 g of anhydrous or 188.13 g of crystallized citric acid in approximately 700 cc of $H_2O$; nearly neutralize with strong $NH_4OH$; cool; measure the volume of the soln or make it up to a convenient volume, taking care to keep the density above 1.09; and make exactly neutral, testing as follows:

With a pipet transfer 5 cc of the citrate soln to a test tube ($7 \times \frac{7}{8}$ inches is a convenient size) and dilute to 20 cc with $H_2O$. Add from a dropping bottle 5 drops of a 0.08% soln of phenol red indicator, either an alcoholic soln of the dye or an aqueous soln of its alkali salt being suitable. From a buret run in approximately 2 $N$ $NH_4OH$ until the color approximates that of a standard buffer soln having a pH of 7.0 contained in a similar test tube and with the same concentration of indicator. (This soln may be prepared by mixing 50 cc of 0.2 $M$ $KH_2PO_4$ soln and 29.54 cc of 0.2 $N$ NaOH soln and making up to 200 cc.[3] Chemicals especially purified for this purpose should be employed, and the standard soln finally used should not stand more than a few days unless some method of checking its reaction is available. The colorimetric $p$H standard solns obtainable from chemical supply houses may be used. These standards will retain their accuracy for an indefinite period if they are protected from excessive exposure to light and heat.) Complete the process by adding the approximately 2 $N$ $NH_4OH$ in small quantities and comparing the colors in a comparator.[4] From the quantity of $NH_4OH$ soln required to produce in the sample a color that exactly matches that of the standard, calculate the quantity required to neutralize the rest of the soln. Add this calculated quantity of $NH_4OH$ to the original soln and check its reaction against that of the neutral standard, using the technic described above. When the colors match dilute the soln to a density of 1.09 at 20°.

The other reagents and solns used are described under **5** and **8.**

### 14 DETERMINATION

(a) *Acidulated samples.*—Heat 100 cc of the $NH_4$-citrate soln to 65° in a 250 cc flask placed in a water bath, keeping the flask loosely stoppered to prevent evaporation. Keep the level of the water in the bath above that of the citrate soln in the flask. When the temp. of the citrate soln has reached 65°, drop into it the filter containing the residue from the water-soluble $P_2O_5$ soln under **11**; close the flask tightly with a smooth rubber stopper; and shake vigorously until the filter paper is reduced to a pulp, relieving the pressure by momentarily removing the stopper. Return the flask to the bath and maintain its contents at exactly 65°. Shake the flask every 5 min.

At the expiration of exactly 30 min.* from the time the filter and the residue were introduced, remove the flask from the bath and immediately filter the contents as rapidly as possible thru a quick-acting filter. Wash with $H_2O$ at 65° until the volume of the filtrate is about 350 cc, allowing time for thoro draining before adding new portions of $H_2O$. Determine the $P_2O_5$ in the citrate-insoluble residue by one of the following methods: (1) Dry the filter and its contents, transfer to a crucible, ignite until all organic matter is destroyed, and digest with 10–15 cc of HCl until all phosphate is dissolved; (2) transfer the wet filter with contents to a 200 cc flask, add 30–35 cc of $HNO_3$ and 5–10 cc of HCl, and boil until all phosphate is dissolved; or (3) treat the filter and its contents as directed under **6** (**c**) or (**d**). Dilute the soln to 200 cc, mix well, filter thru a dry filter, and proceed as directed under **7** or **10**.

(**b**) *Non-acidulated samples.*—In case a determination of citrate-insoluble $P_2O_5$ is required in non-acidulated samples, treat 2 g† of the phosphatic material without previous washing with water as directed under (**a**) and determine $P_2O_5$ as directed under **7** or **10**. If the substance contains much animal matter (bone, fish, etc.), dissolve the residue insoluble in $NH_4$-citrate by one of the processes described under **6** (**c**) or (**d**).

(**c**) *Precipitated phosphates.*[5]—Without washing previously with $H_2O$ treat 1 g of the material as directed under (**a**).

### 15 CITRATE-SOLUBLE PHOSPHORIC ACID—OFFICIAL

Subtract the sum of the water-soluble and citrate-insoluble $P_2O_5$ from the total to obtain the citrate-soluble $P_2O_5$.

### 16 DETECTION OF NITRATES—OFFICIAL

Mix 5 g of the fertilizer with 25 cc of hot $H_2O$, and filter. To 1 volume of this soln add 2 volumes of $H_2SO_4$, free from $HNO_3$ and oxides of N, and allow the mixture to cool. Add a few drops of a concentrated soln of ferrous sulfate in such a manner that the fluids will not mix. If nitrates are present, the junction shows at first a purple, afterwards a brown, color or if only a very minute quantity is present, a reddish color. To another portion of the soln add 1 cc of a 1% soln of $NaNO_3$ and test as before to determine whether sufficient $H_2SO_4$ was added in the first test.

## ORGANIC AND AMMONIACAL NITROGEN ONLY

### *Kjeldahl Method—Official*

### 17 REAGENTS

For ordinary work 0.5 *N* acid is recommended; for work in determining very small quantities of N 0.1 *N* acid is recommended. In titrating mineral acids against a soln of $NH_4OH$ use cochineal or methyl red as indicator.

(**a**) *Standard hydrochloric acid.*—Determine the absolute strength as follows:

PRELIMINARY TEST: Place a measured portion of the acid to be standardized in an Erlenmeyer flask and add an excess of $CaCO_3$ to neutralize free acid and a few drops of a 10% soln of $K_2CrO_4$ as indicator. Titrate with 0.1 *N* $AgNO_3$ soln and note the exact quantity required to precipitate the chlorides.

FINAL DETERMINATION: To a measured portion of the acid to be standardized add from a buret 1 drop in excess of the required quantity of $AgNO_3$ soln as determined by the preliminary test. Heat the mixture to boiling, protect from the light, and allow to stand until the precipitate is granular. Filter on a Gooch crucible, previously heated to 140–150° and weighed; wash with hot $H_2O$, testing the filtrate to verify an excess of $AgNO_3$. Dry the AgCl at 140–150°, cool, and weigh.

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* Digestion for "1 hour" (instead of "exactly 30 min." as here given) was approved as official, first action. See footnote under sec. 11.

† The use of 1 g (instead of 2 g as here given) was approved as official, first action, at the 1930 meeting.

**(b)** *Standard sulfuric acid.*—Determine the strength of the acid by precipitation with $BaCl_2$ soln as follows: Dilute a measured quantity of the acid to be standardized to approximately 100 cc; heat to boiling; and add, dropwise, a 10% soln of $BaCl_2$ until no further precipitation occurs. Continue the boiling for about 5 min., allow to stand for 5 hours or longer in a warm place, pour the supernatant liquid on a weighed Gooch crucible or an ashless filter, treat the precipitate with 25–30 cc of boiling $H_2O$, transfer to the filter, and wash with boiling $H_2O$ until the filtrate is free from chlorides. Dry, ignite over a Bunsen burner, and weigh as $BaSO_4$.

**(c)** *Standard alkali soln.*—A 0.1 *N* soln is recommended. Accurately determine the strength of this soln by titration against the standard acid prepared as directed under **(a)** or **(b)**, or proceed as follows:[16] Place a weighed quantity of pure anhydrous benzoic acid (Bureau of Standards Sample No. 39 is recommended), in a 300 cc flask that has been swept free from $CO_2$; add 20 cc of alcohol, stopper, and let stand until the sample has dissolved; add 3 drops of a 1% soln of phenolphthalein and titrate directly with the alkali soln, taking precautions to prevent the entrance of $CO_2$ into the flask. The effect of the alcohol on the end point should be determined by a blank titration. It is important that the alkali soln be free from $CO_2$. Such a soln may be prepared by dissolving 100 g of NaOH in 100 cc of distilled $H_2O$ and allowing the soln to stand in a stoppered flask until the supernatant liquid is clear. The required quantity of NaOH is then removed by means of a pipet and quickly diluted to the proper volume with $CO_2$-free distilled $H_2O$. This should be protected from $CO_2$ by means of a suitable soda-lime guard tube. An alkali soln that has been standardized in this way may be used in place of the procedures described under **(a)** and **(b)** for the standardization of the hydrochloric and sulfuric acid solns.

**(d)** *Sulfuric acid.*—Should contain 93–96% $H_2SO_4$ and be free from nitrates and $(NH_4)_2SO_4$.

**(e)** *Metallic mercury, or mercuric oxide.*—Should be prepared in the wet way, but not from $Hg(NO_3)_2$.

**(f)** *Copper sulfate.*—Crystallized.

**(g)** *Granulated zinc or pumice stone.*—Add to the contents of the distillation flask to prevent bumping.

**(h)** *Sulfide, or thiosulfate soln.*—Dissolve 40 g of commercial $K_2S$ in 1 liter of $H_2O$. A soln of 40 g of $Na_2S$ or 80 g of $Na_2S_2O_3$, $5H_2O$ in a liter may be used.

**(i)** *Sodium hydroxide soln.*—Dissolve approximately 450 g of commercial NaOH free from nitrates, in 1 liter of $H_2O$. This soln should have a sp. gr. of 1.43–1.48.

**(j)** *Cochineal indicator.*—Digest 3 g of pulverized cochineal in a mixture of 50 cc of 95% alcohol and 200 cc of $H_2O$ for 1 or 2 days at ordinary temp., agitating frequently, and then filter.

**(k)** *Methyl red indicator.*—Dissolve 1 g of methyl red (dimethylaminoazobenzene-orthocarboxylic acid) in 50 cc of 95% alcohol, dilute to 100 cc with $H_2O$ and filter if necessary.

Test reagents before using by a blank determination with sugar. The sugar insures partial reduction of any nitrates present.

### **18** APPARATUS

**(a)** *Kjeldahl flasks for both digestion and distillation.*—Total capacity of about 550 cc, made of hard, moderately thick, and well-annealed glass.

(b) *Distillation flask.*—Use any suitable flask of about 550 cc capacity, fitted with a rubber stopper thru which passes the lower end of a Kjeldahl connecting bulb to prevent NaOH being carried over mechanically during the distillation. Use a bulb about 3 cm in diameter, and tubes of the same diameter as the condenser tube, with which the upper end of the bulb tube is connected by means of rubber tubing.

### 19 DETERMINATION

Place 0.7–3.5 g, according to the N content, of the substance to be analyzed in a digestion flask. Add approximately 0.7 g of HgO, or its equivalent in metallic Hg, and 20–30 cc of $H_2SO_4$ (0.1–0.3 g of crystallized $CuSO_4$ may also be used in addition to the Hg, or in many cases, in place of it). Place the flask in an inclined position and heat below the boiling point of the acid until frothing has ceased. (A small piece of paraffin may be added to prevent extreme foaming.) Increase the heat until the acid boils briskly and digest for a time after the mixture is colorless or nearly so, or until oxidation is complete. (The digestion usually requires at least 2 hours.)

After cooling, dilute with about 200 cc of $H_2O$, and add a few pieces of granulated Zn or pumice stone to prevent bumping, and 25 cc of the $K_2S$ or $Na_2S$ or $Na_2S_2O_3$ soln with shaking. (If $Na_2S_2O_3$ is to be used, it should first be mixed with the NaOH so that they may be added together. When no Hg or HgO is used the addition of $K_2S$ or $Na_2S$ or $Na_2S_2O_3$ soln is unnecessary.) Next add sufficient NaOH soln to make the reaction strongly alkaline (50 cc is usually sufficient), pouring it down the side of the flask so that it does not mix at once with the acid soln. Connect the flask to the condenser by means of a Kjeldahl connecting bulb, taking care that the tip of the condenser extends below the surface of the standard acid in the receiver; mix the contents by shaking; and distil until all $NH_3$ has passed over into a measured quantity of the standard acid. (The first 150 cc of the distillate will generally contain all the $NH_3$.) Titrate with standard alkali soln, using the methyl red or cochineal indicator.

## *Gunning Method—Official*

### 20 REAGENTS

*Potassium sulfate, or anhydrous sodium sulfate.*—Pulverized. The other reagents and solns used are described under **17**.

### 21 APPARATUS

Use the apparatus described under **18**.

### 22 DETERMINATION

Place 0.7–3.5 g, according to the N content, of the substance to be analyzed in a digestion flask. Add 10 g of powdered $K_2SO_4$ or anhydrous $Na_2SO_4$ and 15–25 cc (ordinarily about 20 cc) of concentrated $H_2SO_4$ (0.1 -0.3 g of crystallized $CuSO_4$ may also be added). Conduct the digestion as in the Kjeldahl process, starting with a temp. below the boiling point and increasing the heat gradually until frothing ceases. Digest for a time after the mixture is colorless or nearly so, or until oxidation is complete. (The digestion usually requires at least 2 hours.) Complete as directed under **19**, but do not add $K_2S$ or $Na_2S$ or $Na_2S_2O_3$. In making the mixture alkaline before distilling, add litmus paper or a few drops of phenolphthalein indicator. (The pink color given by phenolphthalein, indicating an alkaline reaction, is destroyed by a considerable excess of strong fixed alkali.)

### *Kjeldahl-Gunning-Arnold Method—Official*

#### 23 REAGENTS AND APPARATUS

Use the apparatus, reagents, and solns described under **17**, **18**, and **20**.

#### 24 DETERMINATION

Place 0.7–3.5 g, according to the N content, of the substance to be analyzed in a digestion flask. Add 15–18 g of $K_2SO_4$ or anhydrous $Na_2SO_4$, 1 g of $CuSO_4$ or approximately 0.7 g of HgO (or its equivalent in metallic Hg), and 25 cc of $H_2SO_4$. Heat the mixture gently until frothing ceases; then boil briskly and continue the digestion for a time after the mixture is colorless or nearly so, or until oxidation is complete. (The digestion usually requires at least 2 hours.) Cool; add about 200 cc of $H_2O$, and if HgO or metallic Hg has been used, add also 50 cc of the $K_2S$ or $Na_2S$ or $Na_2S_2O_3$ soln; make strongly alkaline with the NaOH; and complete as directed under **19**.

## TOTAL NITROGEN

### *Kjeldahl Method Modified to Include the Nitrogen of Nitrates—Official*

#### 25 REAGENTS

(**a**) *Zinc dust.*—An impalpable powder. Granulated Zn or Zn filings are not satisfactory.

(**b**) *Sodium thiosulfate* ($Na_2S_2O_3 . 5H_2O$).

(**c**) *Commerical salicylic acid.*

The other reagents and solns are described under **17**.

#### 26 APPARATUS

Use the apparatus described under **18**.

#### 27 DETERMINATION

Place 0.7–3.5 g, according to the N content, of the substance to be analyzed in a Kjeldahl digestion flask. (1) Add 30 cc of $H_2SO_4$ containing 1 g of salicylic acid, shake until thoroly mixed, allow to stand for at least 30 min. with frequent shaking or until complete soln results, and then add 5 g of crystallized $Na_2S_2O_3$ and digest as directed below; or, (2) add to the substance 30 cc of $H_2SO_4$ containing 2 g of salicylic acid, allow to stand at least 30 min. with frequent shaking or until complete soln results, and then add gradually 2 g of Zn dust, shaking the contents of the flask at the same time, and digest as follows:

Heat over a low flame until all danger from frothing has passed. Then increase the heat until the acid boils briskly and continue the boiling until white fumes no longer escape from the flask (5–10 min.). Add approximately 0.7 g of HgO, or its equivalent in Hg, and continue the boiling until the liquid in the flask is colorless, or nearly so. If the contents of the flask are likely to become solid before this point is reached, add 10 cc more of $H_2SO_4$. Complete the determination as directed under **19**. Test the reagents by blank determinations.

### *Gunning Method Modified to Include the Nitrogen of Nitrates—Official*

#### 28 REAGENTS AND APPARATUS

Use the apparatus, reagents, and solns described under **17**, **18**, **20**, and **25**.

#### 29 DETERMINATION

Place 0.7–3.5 g, according to the N content, of the substance to be analyzed in a digestion flask. Add 30–35 cc of salicylic acid mixture (1 g of salicylic acid to 30 cc of $H_2SO_4$); shake until thoroly mixed; and allow to stand, shaking frequently, for at least 30 min., or until complete soln results. Add 5 g of $Na_2S_2O_3$ and heat the soln for 5 min.; cool, add 10 g of $K_2SO_4$ or anhydrous $Na_2SO_4$, heat very gently until foaming ceases, and proceed as directed under **22.**

### AMMONIACAL NITROGEN

**30**

### *Magnesium Oxide Method—Official*

Place 0.7–3.5 g, according to the $NH_3$ content, of the substance to be analyzed in a distillation flask with about 200 cc of $H_2O$ and 2 g* or more of MgO, free from carbonates. Connect the flask with a condenser by means of a Kjeldahl connecting bulb, distil 100 cc of the liquid into a measured quantity of standard acid, and titrate with standard alkali, using cochineal or methyl red indicator.

### NITRIC AND AMMONIACAL NITROGEN

**31**

### *Reduced Iron Method—Official*

(Applicable only in the absence of cyanamides and urea)

Place 0.7 or 1 g of the sample in a 500 cc flask, add about 30 cc of $H_2O$ and 2–3 g of reduced Fe, and, after allowing the mixture to stand sufficiently long to insure soln of the soluble nitrates and $NH_4$ salts, add 10 cc of $H_2SO_4$ (1+1). Shake thoroly, place a long-stemmed funnel in the neck of the flask to prevent mechanical loss, and allow to stand until the violence of the reaction has moderated. Heat the soln slowly, boil for 5 min., and cool. Add about 100 cc of $H_2O$, a little paraffin, and 7–10 g of MgO, free or nearly free from carbonates. Connect the flask by means of a Kjeldahl connecting bulb with a condenser, such as is used in the Kjeldahl method, and boil the mixture nearly to dryness (about 40 min.); collect the $NH_3$ in a measured quantity of standard acid; and titrate with standard alkali soln, using cochineal or methyl red indicator. The N obtained represents the nitrates plus the $NH_4$ salts contained in the sample.

In the analysis of nitrate salts proceed as above, but use 25 cc of the nitrate soln, equivalent to 0.25 g of the sample, with 5 g of reduced Fe. After boiling, add 75 cc of $H_2O$ and an excess of NaOH soln and complete the determination as above.

### NITRATE NITROGEN

**32**

### *Robertson Method—Official, First Action*[6]

(Applicable in the presence of cyanamides and urea)

(**a**) Determine the total N as directed under **27** or **29.**

(**b**) Weigh out 2.0 g of the fertilizer mixture on a filter, wash with $H_2O$ to nearly 200 cc in a graduated flask, and make up to volume. Determine the N in the residue as directed under **19, 22,** or **24.**

(**c**) Determine the ammoniacal N in 50 cc of the filtrate as directed under **30.**

(**d**) Place another 50 cc portion of the filtrate in a 500 cc Kjeldahl flask and add 2 g of ferrous sulfate and 20 cc of $H_2SO_4$ (sp. gr. 1.84). (If the total N is over 5%, use 5 g of ferrous sulfate.) Digest over a hot flame until all the $H_2O$ is evaporated and white fumes appear and continue the digestion for at least 10 min. to drive off the nitrate N. If severe bumping occurs, add 10–15 glass beads. Add 0.65 g of Hg or its equivalent of HgO and digest until all the organic matter is oxidized. Cool, dilute, add the $K_2S$ soln, and complete the determinaton as directed under **24.** (A pinch of a mixture of Zn dust and granular Zn (20-mesh) should be added to each flask before distillation to prevent bumping.)

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* "2 g" has been approved as official, first action. The method as printed in the 2nd Edition of this book read "5 grams."

Total N(a)—water-insoluble N(b) = water-soluble N. Water-soluble N – the N obtained in (d) = the nitrate N.

Ammoniacal N + nitrate N = mineral N. Total N – mineral N = organic N.

### **33** *II. Jones Modification of the Robertson Method*

(Applicable when a determination of water-soluble nitrogen is not needed)

Weigh 0.5 g of the sample into a Kjeldahl flask. Add 50 cc of $H_2O$ and rotate gently; then add 2 g of ferrous sulfate and rotate. Add 20 cc of $H_2SO_4$ (sp. gr. 1.84). Digest over a hot flame. When the water is evaporated and white fumes appear, add 0.65 g of Hg and complete the digestion as in the regular Kjeldahl method. Cool, dilute, and distil as usual. The total N – the N thus found = the nitrate N.

### NITROGEN IN NITRATE SALTS

### **34** *I. Ferrous Sulfate-Zinc-Soda Method—Official*

Place 0.35, 0.5, or 0.7 g of the nitrate salt in a 600–700 cc flask and add 200 cc of $H_2O$, 5 g of powdered Zn, 1–2 g of ferrous sulfate, and 50 cc of NaOH soln (sp. gr. 1.33). Connect with the distilling apparatus, distil, collect the distillate in the usual way in 0.1 *N* $H_2SO_4$, and titrate with standard alkali, using cochineal or methyl red indicator.

### **35** *II. Devarda Method[7]—Official, First Action*

Place 0.5 g of the nitrate salt in a 600–700 cc flask and add 300 cc of $H_2O$, 3 g of the Devarda alloy[8] and 5 cc of NaOH soln (42% by weight), pouring the latter down the side of the flask so that it does not mix at once with the contents. Connect, by means of a Davisson[9] or other suitable scrubbing bulb that will prevent the passing over of any portion of the spray, with a condenser, the tip of which always extends beneath the surface of the standard acid in the receiving flask. Mix the contents of the distilling flask by rotating. Heat slowly at first and then at such a rate that the 250 cc of distillate required will pass over in 1 hour. Collect the distillate in a measured quantity of standard acid and titrate with standard alkali soln, using cochineal or methyl red indicator.

## NITROGEN ACTIVITY METHODS

### WATER-INSOLUBLE ORGANIC NITROGEN SOLUBLE IN NEUTRAL PERMANGANATE—OFFICIAL

### **36** *Preliminary Test (Determination of Water-Insoluble Organic Nitrogen)*

Place 1 or 1.4 g of the material on an 11 cm filter paper and wash with $H_2O$ at room temp. until the filtrate measures 250 cc. Dry, and determine N in the residue as directed under **19** or **22**, making a correction for water-insoluble N of the filter paper, if necessary.

### **37** DETERMINATION

Place the quantity of fertilizer equivalent to 50 mg of water-insoluble organic N, as determined under **36**, on a moistened 11 cm filter paper and wash with $H_2O$ at room temp. until the filtrate measures 250 cc. Transfer the insoluble residue with 25 cc of tepid $H_2O$ to a 400 cc Griffin low-form beaker, add 1 g of $Na_2CO_3$, mix, and add 100 cc of a 2% soln of $KMnO_4$. Cover with a watch-glass and immerse for 30 min. in a steam or hot water bath, keeping the liquid in the beaker below that of the $H_2O$ in the bath. Stir twice at intervals of 10 min. At the end of 30 min. remove from the bath, add immediately 100 cc of cold $H_2O$, and filter thru a heavy 15 cm folded filter. Wash with small quantities of cold $H_2O$ until the filtrate measures about 400 cc. Determine N in the residue and filter, as directed under **19** or **22**, correcting for the N contained in the filter. The N thus obtained is the inactive water-insoluble organic N. The N obtained under **36** −the percentage of N found = the water-insoluble organic N soluble in neutral permanganate.

## WATER-INSOLUBLE ORGANIC NITROGEN DISTILLED FROM ALKALINE PERMANGANATE—OFFICIAL

### 38 REAGENTS

(**a**) *Stock soln of potassium permanganate.*—Dissolve 50 g of $KMnO_4$ in a liter of $H_2O$. Dissolve 0.5 g of Na-oxalate in 300 cc of $H_2O$ and 10 cc of concentrated $H_2SO_4$. Heat to 75–80° and titrate with the $KMnO_4$ soln, using a Mohr pipet or an all-glass buret to contain the permanganate soln. $235.89 \div$ by the result of the titration in cc = the concentration of $KMnO_4$ in grams per liter. Adjust the concentration to 50 g per liter, protect from the light, and store at a temp. above 15°.

(**b**) *Stock soln of sodium hydroxide.*—Dissolve 300 g of NaOH in a liter of $H_2O$. Cool before using.

(**c**) *Alkaline permanganate soln.*—Mix equal quantities of the stock solns (**a**) and (**b**) and add 10 cc of $H_2O$ for each liter of soln that the mixture is calculated to make. Use this soln immediately, as it is unstable.

### 39 PREPARATION OF SAMPLE

(**a**) *Mixed fertilizers.*—Place the quantity of material equivalent to 50 mg of water-insoluble organic N, as determined under **36**, on a filter paper and wash with $H_2O$ at room temp. until the filtrate measures 250 cc.

When it is found necessary to use 4 g or more of the original material, weigh the required quantity into a small beaker, wash by decantation, finally transfer to the filter, and finish the extraction as previously directed.

(**b**) *Raw materials.*—Place the quantity of material equivalent to 50 mg of water-insoluble organic N, as determined under **36**, in a small mortar; add about 2 g of powdered rock phosphate, mix thoroly, transfer to a filter paper, and wash with water at room temp. until the filtrate measures 250 cc. If much oil or fat is present, it is well to wash with ether before extracting with water.

### 40 DETERMINATION[10]

Dry the residue remaining after treatment of the material as described in **39** at a temp. not exceeding 80° and transfer from the filter to a 500–600 cc Kjeldahl distillation flask, loosening adhering particles by rubbing gently with a stiff brush but avoiding the transfer of portions of the brush or of paper fibers. Add 20 cc of $H_2O$, 15–20 small glass beads or fragments of pumice stone, a drop of mineral lubricating oil weighing not more than 50 mg, and 100 cc of alkaline permanganate soln. Connect with an upright condenser to the lower end of which has been attached a 100 cc graduated cylinder containing standard acid and so arranged as to receive the distillate below the surface of the acid or otherwise trapped so as to prevent loss of $NH_3$ fumes. Digest slowly with a very low flame for 30 min., barely below distillation point, using coarse wire gauze and asbestos paper between the flask and flame. Gradually raise the temp. and, after all danger from frothing has passed, distil 95 cc in 60 min. (plus or minus 5 min.), controlling the distillation to obtain approximately 24 cc of distillate in each 15 min. period. Conduct the first part of the distillation over a bare flame but use wire gauze 10 min. before completion to avoid breaking the flask. Transfer the distillate to an Erlenmeyer flask or to a beaker and titrate with standard alkali, using cochineal or methyl red indicator. When a tendency to froth is noticed, lengthen the digestion period, and no trouble will be experienced when the distillation is begun. During the digestion gently rotate the flask occasionally, particularly if the material shows a tendency to adhere to the sides.

The N thus obtained is the active water-insoluble organic N. If active water-insoluble N is found to be less than 55% of the total water-insoluble organic N present, it is recommended that a second portion of the sample be prepared as directed under **39**(a). Dry the residue below 80°, transfer from the filter to a Kjeldahl flask as directed above, and determine the N as directed under **19** or **22**. Recalculate the percentage of active water-insoluble N on the basis of the quantity of water-insoluble N thus found.

Previous to digestion with alkaline permanganate, the washed sample may be transferred from the filter to the flask by spreading the filter on a metal disk bent to form a trough that fits the palm of the hand, brushing the larger portion of the material into the flask with a spatula, and washing in the remainder with 20 cc of $H_2O$ from a 20 cc pipet or small wash bottle. Do not add more $H_2O$ before the digestion with alkaline permangate, but, with this exception, proceed as with the transfer of the dried material.

## POTASH

### *Method I. Lindo-Gladding*[11]*—Official*

### **41** REAGENTS

**(a)** *Ammonium chloride soln.*—Dissolve 100 g of $NH_4Cl$ in 500 cc of $H_2O$, add 5–10 g of pulverized $K_2PtCl_6$, and shake at intervals for 6–8 hours. Allow the mixture to settle overnight and filter. The residue may be used for the preparation of a fresh supply.

**(b)** *Platinum soln.*—A $PtCl_4$ soln containing the equivalent of 1 g of metallic Pt (2.1 g of $H_2PtCl_6$) in every 10 cc. For materials containing less than 15% of $K_2O$, a $PtCl_4$ soln containing 0.2 g of metallic Pt (0.42 g of $H_2PtCl_6$) in each 10 cc is recommended.

**(c)** 80% *alcohol.*—Sp. gr. 0.8593 at 20/4°.

### **42** PREPARATION OF SOLUTION

**(a)** *Mixed fertilizers.*—Place 2.5 g of the sample upon a 12.5 cm filter paper and wash with successive small portions of boiling $H_2O$ into a 250 cc volumetric flask until the filtrate amounts to about 200 cc. Add to the hot soln a slight excess of $NH_4OH$ and sufficient saturated $NH_4$-oxalate soln to precipitate all the lime present, cool, dilute to 250 cc, mix, and pass thru a dry filter.

**(b)** *Potash salts; muriate and sulfate of potash, sulfate of potash and magnesia; and kainit.*—Dissolve 2.5 g and dilute to 250 cc without the addition of $NH_4OH$ and $NH_4$-oxalate.

**(c)** *Organic compounds.*—When it is desired to determine the total amount of $K_2O$ in organic substances, such as cottonseed meal, tobacco stems, etc., saturate 10 g of the sample with $H_2SO_4$ and ignite in a muffle at a low red heat to destroy organic matter. Add a little HCl, warm slightly in order to loosen the mass from the dish, transfer to a 500 cc volumetric flash, add $NH_4OH$ and saturated $NH_4$-oxalate soln, cool, dilute to 500 cc, mix, pass thru a dry filter, and proceed as directed under **43**(a).

(d) *Ashes from wood, cotton hulls, etc.*—Boil 10 g of the sample with 300 cc of $H_2O$ for 30 min., and add to the hot soln a slight excess of $NH_4OH$ and then sufficient saturated $NH_4$-oxalate soln to precipitate all the lime present. Cool, dilute to 500 cc, mix, and pass thru a dry filter.

### **43** DETERMINATION

(a) *Mixed Fertilizers.*—Evaporate a 50 cc aliquot of soln **42** (a) nearly to dryness, add 1 cc of $H_2SO_4$ (1+1), evaporate to dryness, and ignite to whiteness. Maintain a full red heat until the residue is perfectly white. Dissolve the residue in hot $H_2O$ using at least 20 cc for each decigram of $K_2O$ present, and add a few drops of HCl and then an excess of the Pt soln. Evaporate on a water bath to a thick paste, avoiding exposure to $NH_3$. Treat the residue with the 80% alcohol. Filter on a Gooch crucible and wash the precipitate thoroly with 80% alcohol, both by decantation and on the filter, continuing the washing after the filtrate is colorless. Then wash 5 or 6 times with 10 cc portions of the $NH_4Cl$ soln to remove impurities from the precipitate. Wash again thoroly with the 80% alcohol and dry the precipitate for 30 min. at 100°. Weigh and calculate to $K_2O$. The precipitate should be completely soluble in water.

(b) *Muriate of potash.*—Acidify 50 cc of the soln prepared according to **42** (b) with a few drops of HCl, add 10 cc of the Pt soln, and evaporate to a thick paste. Treat the residue as directed under (a).

(c) *Sulfate of potash, sulfate of potash and magnesia, and kainit.*—Acidify 50 cc of the soln prepared according to **42**(b) with a few drops of HCl and add 15 cc of the Pt soln. Evaporate the mixture and proceed as directed under (a), but use 25 cc portions of the $NH_4Cl$ soln.

(d) *Water-soluble potash in ashes from wood, cotton hulls, etc.*—Prepare the soln according to **42**(d) and proceed as directed under (a), paying special attention to the last sentence.

For the conversion of $K_2PtCl_6$ to KCl use the factor 0.3065; to $K_2SO_4$, 0.35843; to $K_2O$, 0.19376.

## *Method II—Official*

(In the presence of soluble sulfates use preferably Method I.)

### **44** REAGENTS

Use the reagents and solns described under **41.**

### **45** PREPARATION OF SOLUTION

Prepare the soln as directed under **42**, omitting in all cases the addition of $NH_4OH$ and $NH_4$-oxalate.

### **46** DETERMINATION

Dilute 25 cc of the soln prepared as directed under **45** (50 cc if less than 10% of $K_2O$ is present) to 150 cc; heat to 100°; and add, drop by drop, with constant stirring, a slight excess of a 10% $BaCl_2$ soln. Without filtering, add in the same manner an excess of a saturated soln of $Ba(OH)_2$. Filter while hot and wash until the precipitate is free from chlorides. Add to the filtrate 1 cc of strong $NH_4OH$, and then a saturated soln of $(NH_4)_2CO_3$ until the excess of Ba is precipitated. Heat, and add, in fine powder, 0.5 g of pure oxalic acid or 0.75 g of $NH_4$-oxalate. Filter, wash free from chlorides, evaporate the filtrate to dryness in a Pt dish, and ignite carefully over the free flame below a red heat until all volatile matter is driven off. Digest the residue with hot water, filter thru a small filter; and dilute the filtrate, if necessary, to provide for each decigram of $K_2O$ at least 20 cc of liquid. Acidify with a few drops of HCl and add an excess of the Pt soln. Evaporate on a water bath to a thick paste; treat the residue repeatedly with 80% alcohol, decanting thru a weighed Gooch crucible or other form of filter; transfer the precipitate to the filter; and wash thoroly with the 80% alcohol. Dry for 30 min. at 100° and weigh. If there is an appearance of foreign matter in the double salt, wash as directed under **43**(a) with several portions of 10 cc each of the $NH_4Cl$ soln.

## THOMAS OR BASIC SLAG

### 47 MECHANICAL ANALYSIS—OFFICIAL

Proceed as directed under **3**, using 10 g of material.

### 48 PREPARATION OF SAMPLE—OFFICIAL

Proceed as directed under **2**.

### TOTAL PHOSPHORIC ACID

### *Gravimetric Method—Official*

### 49 PREPARATION OF SOLUTION

Prepare the soln for analysis as directed under **6**(b), or in HCl alone. In the latter case, measure out the portion for analysis, add 3–5 cc of $HNO_3$, and heat for a few min.

### 50 DETERMINATION

Dehydrate an aliquot (20 cc) of the soln, obtained as directed under **49**, by evaporating to dryness on a steam or hot water bath; treat with 5 cc of HCl and 25 cc of hot $H_2O$; digest in order to complete soln; and filter off $SiO_2$. From this point proceed as directed under **7**. Before precipitating with magnesia mixture, add 5 cc of a 5% Na-acetate soln.

### 51 *Volumetric Method—Official*

Prepare the soln as directed under **6**(b) and determine the $P_2O_5$ in an aliquot of this soln as directed under **10**, standardizing the solns against a standard phosphate material of approximately the same composition as the sample under examination

### CITRIC ACID-SOLUBLE PHOSPHORIC ACID

### *Gravimetric Method—Official*

### 52 PREPARATION OF SOLUTION

Weigh 5 g of the slag, prepared as directed under **2**, into a 500 cc Wagner flask containing 5 cc of 95% alcohol. (The neck of the flask should have a width of at least 22 mm, and the graduation marks should be at least 8 cm below the mouth.) Make up to the mark with 2% citric acid soln of a temp. of 17.5°. Fit the flask with a rubber stopper and place at once in a rotary apparatus, shaking the flask for 30 min. at the rate of 30–40 r.p.m. At the end of this time remove the flask, filter immediately on a dry filter, and analyze the soln at once.

### 53 DETERMINATION

To 50 cc of the clear filtrate in a beaker add 100 cc of molybdate soln prepared as directed under **5**(a), and place the beaker in a water bath; when the temp. of the contents reaches 65°, remove and cool to room temp. Filter and wash the yellow precipitate of $NH_4$-phosphomolybdate 4 or 5 times with 1% $HNO_3$. Dissolve the precipitate in 100 cc of cold 2% $NH_4OH$; nearly neutralize with HCl; add to the soln drop by drop, with continuous stirring, 15 cc of magnesia mixture prepared as directed under **5**(**c**); and proceed as directed under **7**.

**54** *Volumetric Method—Official*

In an aliquot of the clear soln, prepared as directed under **52**, determine the $P_2O_5$ as directed under **10**.

## SPECIAL METHODS

### BORIC ACID

#### WATER-SOLUBLE BORIC ACID[12]—OFFICIAL

**55** REAGENTS

(**a**) *Barium chloride soln.*—Dissolve 10 g of $BaCl_2.2H_2O$ in $H_2O$ and dilute to 100 cc.

(**b**) *Barium hydroxide.*—Powdered.

(**c**) *Standard boric acid soln.*—0.1 *N*.

(**d**) *Standard sodium hydroxide soln.*—Prepare this soln free from carbonates by first making a saturated soln (100 g of NaOH in 100 cc of $H_2O$) in order to precipitate any $Na_2CO_3$ present when the soln is allowed to stand in a vessel from which the $CO_2$ of the air is excluded. Filter thru a hard filter that has been soaked in alcohol; dilute a portion with boiled and cooled $H_2O$ to about 0.1 *N*; and accurately determine the strength of the soln by titration, as described under **56**(**a**), against the standard boric acid.

(**e**) *Hydrochloric acid soln.*—(1) Approximately 0.1 *N* and (2) approximately 0.5 *N* soln.

(**f**) *Neutral mannitol* (*mannite*).

(**g**) *Methyl red indicator.*—Dissolve 0.1 g of methyl red in 50 cc of 95% alcohol, dilute to 1 liter, and filter, if necessary.

(**h**) *Phenolphthalein indicator.*—Prepare as directed under **8**(**d**).

**56** DETERMINATION

(**a**) *Mineral salts.*—Dissolve 5-10 g of the sample in 50–75 cc of hot $H_2O$; decompose carbonates, if present, with a slight excess of HCl; heat to boiling; and add sufficient 10% $BaCl_2$ soln to precipitate the sulfates, using about 10 cc in excess. Next add in small quantities sufficient powdered $Ba(OH)_2$ to make the soln alkaline, avoiding a large excess; boil for about 5 min., or until any $NH_3$ present has been expelled; filter; wash into a 300 cc flask; and make acid with HCl, using an excess equivalent to a few cc of 0.1 *N* soln. Boil for 15 min. to expel $CO_2$, cool by placing the flask in cold water, and bring to neutrality by first adding 4 or 5 drops of the methyl red indicator and then the standard NaOH soln until the color of the soln changes from pink to yellow. If the neutral point has been exceeded, or if there is any doubt as to this, restore the pink color by adding a few drops of the approximately 0.1 *N* HCl and change the color to yellow again with the minimum quantity of the standard NaOH soln. Add 1–2 g of neutral mannitol and a few tenths of a cc of phenolphthalein indicator, note the buret reading, and again titrate the soln with the standard NaOH soln until a pink color develops. Add a little more mannitol and if the pink color disappears continue the addition of the standard alkali until it reappears.

Repeat until the addition of mannitol has no further action on the end point. (If the content of boric acid in the soln titrated is low, one addition of mannitol is usually sufficient.) From the volume of the standard alkali required in the titration after the addition of the mannitol, corrected for the volume required when running a blank, calculate the quantity of borax in the sample. 1 cc. of 0.1 $N$ NaOH soln $=0.0062$ g of boric acid, or 0.00503 g of anhydrous borax.

When an acid soln of the sample to be analyzed gives no precipitate upon the addition of a soln of $CaCl_2$ and sufficient $NH_4OH$ to give an alkaline reaction, phosphates and Fe and Al salts are absent, and that portion of the determination which involves treatment with $BaCl_2$ and $Ba(OH)_2$ for the removal of these constituents may be omitted.

**(b)** *Mixed fertilizers and organic compounds.*—Weigh 5 g of the sample into a 250 cc beaker, add 50 cc of hot $H_2O$, cover with a watch-glass, digest for 15–20 min. on a water bath, filter, and wash into another beaker of the same capacity. Heat the filtrate to boiling and add 15 cc of $BaCl_2$ soln followed without undue loss of time by sufficient powdered $Ba(OH)_2$ to give an alkaline reaction as indicated by phenolphthalein; boil for about 5 min., gently to prevent frothing over; filter; and wash. Or, if preferred, make up to the mark in a volumetric flask and take an aliquot. Evaporate the filtrate or aliquot to dryness in a Pt or porcelain dish and ignite the residue (preferably in a muffle furnace at a temp. just below redness) until the organic matter is completely carbonized. Treat the ignited residue with hot $H_2O$, make slightly acid with HCl, heat nearly to boiling, make alkaline again with a slight excess of $Ba(OH)_2$, and filter into a 300 cc flask. Acidify with HCl $(1+9)$, using an excess equivalent to a few cc of a 0.1 $N$ soln, boil to expel $CO_2$, and titrate as directed under **(a)**.

If the $Ba(OH)_2$ has been added in slight excess only there is a tendency for the filtrate to become acid during evaporation with a possible loss of borax. It is important, therefore, that the soln be kept alkaline by repeated additions of $Ba(OH)_2$, if necessary, until the evaporation is completed.

If the filtrate from the $BaCl_2$-$Ba(OH)_2$ precipitate is titrated in this determination before the soluble organic matter is destroyed, the end points in the titration will usually be too indefinite to give accurate results. The purpose in evaporating the filtrate and igniting the residue, therefore, is to get rid of soluble organic constituents which interfere with the titration. When the sample contains a relatively high boric acid content, in excess of 0.5%, a smaller sample may be taken and the quantity of organic matter present may then be too small to interfere seriously with the sharpness of the end points during the titration. When such is the case, boil the soln after the addition of the $Ba(OH)_2$ until any $NH_3$ present has been expelled. Omit evaporating the filtrate from the $BaCl_2$-$Ba(OH)_2$ precipitate. Add to the filtrate an excess of HCl equivalent to a few cc of a 0.1 $N$ soln, boil to expel $CO_2$, and titrate as directed under the determination of mineral salts.

## ACID-SOLUBLE BORIC ACID[13]—OFFICIAL

### 57 REAGENTS

**(a)** *Phosphoric acid* ($H_3PO_4$)—85%.

**(b)** *Methyl alcohol.*—At least 95%.

**(c)** *Hydrochloric acid.*—Approximately 1 $N$.

The other reagents and solns used are described under **55**.

### 58 APPARATUS

The apparatus[14] consists of two 200 cc round-bottomed flasks, a Liebig condenser, and a 200 cc Erlenmeyer receiving flask. One of the 200 cc round-bottomed flasks, No. 2, is supplied with a doubly perforated rubber stopper, thru one hole of which passes a glass tube running to the bottom of the flask; thru the other hole is a short tube leading to the condenser. The other 200 cc round-bottomed flask, No. 1, is fitted with a perforated rubber stopper and a short bent tube connected by rubber tubing with the long tube in flask No. 2. The whole apparatus is supported by clamps and rings on two stands.

### 59 DETERMINATION

If the material to be examined is a mixed fertilizer or probably contains less than 2% of anhydrous borax, weigh 5 g into flask No. 2. If the material is a chemical containing much more than 2% of borax, use 2 g. Then add 5 cc of the 85% $H_3PO_4$ and 20 cc of methyl alcohol and connect the flask with the condenser. Add 100 cc of methyl alcohol to flask No. 1, which is set in a water bath and connected with flask No. 2. Place the receiving flask in position at the end of the condenser and apply sufficient heat to the water bath to keep a steady flow of bubbles of methyl alcohol passing thru flask No. 2. Also apply some heat to flask No. 2 to keep the volume at about 25 cc. Continue the distillation until 100 cc of distillate is obtained, which should require about 30 min. When distillation is complete, add 2 or 3 drops of phenolphthalein indicator to the distillate and 5–10 cc of 0.1 *N* NaOH, or enough to give it a permanent pink color. Stopper the flask, shake well, and connect at once with a condenser by means of a Hopkins or similar bulb; distil off the alcohol, and save for another determination. Use a water bath, not a gas burner for this purpose. Transfer the residue, which should be not less than 10 cc, to a Pt or porcelain dish, using as little $H_2O$ as possible, and evaporate to dryness on a steam or water bath. When dry, ignite below redness. Then acidify with a few drops of approximately 1 *N* HCl, add 20–25 cc of $H_2O$, and warm for 1–2 min. on a steam bath. Filter into a small flask, thoroly wash, dilute to about 50–75 cc, attach to an air-cooled condenser, and boil gently for a few min. to remove $CO_2$. Add 3 or 4 drops of the methyl red indicator and then the 0.1 *N* NaOH until the red color just disappears. Add about 1 g of mannitol, or less if but a small amount of boron is present. (At this point, if boric acid is present, the soln will take on a pinkish color; the depth of color depends on the quantity present, 0. 01 or 0.02% usually being sufficient to give the color if the soln has been carefully neutralized with the NaOH soln.) Then add 2 or 3 drops of phenolphthalein indicator and titrate the soln with the 0.1 *N* NaOH. A blank should be run with the reagents, but if the NaOH is free from $CO_2$ the blank should not be more than 0.2 cc. Calculate to boric acid or anhydrous borax as directed under **56**(a).

## CHLORINE[15]—OFFICIAL, FIRST ACTION

### 60 REAGENTS

(a) *Standard silver nitrate soln.*—Dissolve about 5 g of pure recrystallized $AgNO_3$ in $H_2O$ and dilute to 1 liter. Standardize against pure, dry NaCl and adjust so that 1 cc of the soln is equivalent to 0.001 g of Cl.

(b) *Potassium chromate indicator.*—Dissolve 5 g of $K_2CrO_4$ in 100 cc of $H_2O$.

### 61 DETERMINATION

Place 2.5 g of the sample on an 11 cm filter paper and wash with successive portions of boiling $H_2O$ until the washings amount to nearly 250 cc, collecting the filtrate in a 250 cc volumetric flask. Cool, dilute to the mark with $H_2O$, and mix well. Pipet 50 cc into a 150 cc beaker, add 1 cc of the $K_2CrO_4$ indicator, and titrate with the standard $AgNO_3$ soln until the color produced by $Ag_2CrO_4$ appears as a permanent red.

## SELECTED REFERENCES

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[2] Ibid., **5,** 92 (1921); 443 (1922); **6,** 384 (1923).
[3] Clark, The Determination of Hydrogen Ions, 1928, p. 200.
[4] Ibid., p. 171.
[5] J. Assoc. Official Agr. Chem., **5,** 460 (1922).
[6] Ibid., **13,** 38 (1930).
[7] Chem. Ztg., **16,** 1952 (1892), J. Ind. Eng. Chem., **11,** 306 (1919); **12,** 352 (1920); J. Assoc. Official Agr. Chem., **5,** 450 (1922); **6,** 391 (1923).
[8] J. Ind. Eng. Chem., **11,** 307 (1919).
[9] Ibid., **11,** 465 (1919).
[10] J. Assoc. Official Agr. Chem., **11,** 34 (1928); **13,** 39 (1930).
[11] Ibid., **6,** 399 (1923); **7,** 382 (1924).
[12] Ibid., **5,** 80 (1921); 327 (1922).
[13] J. Am. Chem. Soc., **20,** 288 (1898); J. Assoc. Official Agr. Chem., **5,** 88 (1921).
[14] Leach, Food Inspection and Analysis, 4th ed., 1920, p. 884.
[15] J. Assoc. Official Agr. Chem., **11,** 34 (1928).
[16] Hillebrand and Lundell, Applied Inorganic Analysis, 1929, pp. 139, 142.

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# III. SEWAGE*

* See note at bottom of p. xv.

# IV. AGRICULTURAL LIMING MATERIALS[1]

### 1 DIRECTIONS FOR SAMPLING—TENTATIVE

Take a sample that is representative of the lot or shipment and that does not contain a disproportionate quantity of the surface or of any modified or damaged zone in the following manner:

(a) *Burnt, or lump lime, in bulk.*—Collect a composite sample of not less than 10 shovelfuls per car, with proportionate quantities from smaller lots, taking each shovelful from a different part of the lot or shipment. Crush immediately to pass a circular opening 1 inch in diameter; mix thoroly and rapidly; quarter down to a 5-lb. sample; and place in a properly labeled, dry, air-tight container.

(b) *Burnt, or lump lime, in barrels.*—Select at random 5 barrels from each lot or shipment of 20 tons or less and 1 additional barrel for each additional 5 tons. Take not less than 10 lbs. from each barrel selected and treat as directed under (a).

(c) *Hydrated lime and ground burnt lime, in bags.*—Select 10 bags from different parts of each lot or shipment of 20 tons or less and 1 additional bag for each additional 5 tons. From each of the bags sampled withdraw a core from top to bottom by means of a sampling tube; mix these portions thoroly and rapidly on heavy sized paper or oilcloth; quarter down to a 2-lb. sample; and place in a properly labeled, dry, airtight container.

(d) *Ground limestone and ground marl, in bags.*—Proceed as directed under (c).

(e) *Ground limestone, ground burnt lime, and ground marl, in bulk.*—By means of a slotted sampling tube, withdraw samples to full sampler depth from 10 points in the lot or shipment; mix thoroly and rapidly on heavy sized paper or oilcloth; quarter down to a 2-lb. sample; and place in a properly labeled, dry, air-tight container.

### 2 PREPARATION OF SAMPLE—TENTATIVE

Grind the sample in a porcelain mortar or porcelain ball mill to pass a 60-mesh sieve, mix thoroly, and preserve in an air-tight container.

## NEUTRALIZING VALUE—TENTATIVE

### 3 REAGENTS

(a) *Sodium hydroxide soln.*—0.25 *N*. Prepare free from carbonates and store in a bottle provided with a siphon tube and with guard tubes containing soda-lime, or other suitable device, to prevent absorption of $CO_2$ from the air.

(b) *Nitric acid.*—0.5 *N*. Standardize against (a), using phenolphthalein indicator.

(c) *Phenolphthalein indicator.*—Prepare as directed under **II, 8(d)**.

### 4 DETERMINATION

Place 0.5 g of burnt or hydrated lime (1 g of ground limestone or ground marl), prepared as directed under **2**, in a 150 cc beaker; add 50 cc of the 0.5 *N* $HNO_3$; cover the beaker with a watch-glass; and boil for 5 min. Cool, and titrate the excess of acid with the 0.25 *N* NaOH soln, using phenolphthalein indicator. Calculate and report the results as percentage of CaO in burnt and hydrated lime and as percentage of $CaCO_3$ equivalent for limestones and marls.

## CAUSTIC VALUE[2]—OFFICIAL

### 5 APPARATUS

In this apparatus *A* is a 500 cc Erlenmeyer flask of Pyrex glass, and *F* is a filter cone packed nearly full with cotton, which is covered to a depth of from 2 to 3 mm with lightly compacted macerated filter paper. The filter cone is connected with the syphon tube *B* by means of thick-walled rubber tubing. The receiving flasks *m* and *n* are calibrated to deliver 50 and 100 cc respectively. *S* is a suction flask.

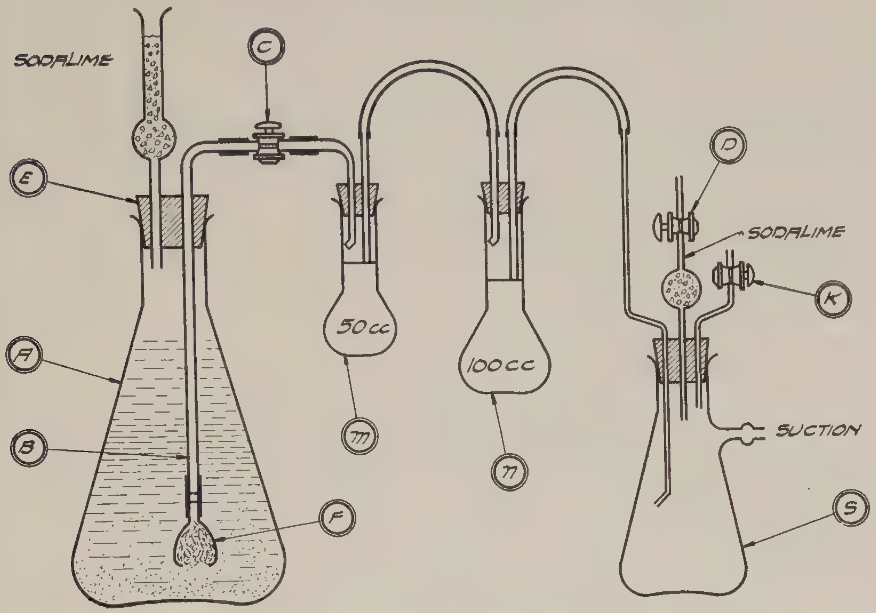

FIG. 3.—APPARATUS FOR AUTOMATIC FILTRATION AND MEASUREMENT OF LIME SOLUTIONS

## 6 DETERMINATION

Grind the lime sample to pass a 100-mesh sieve, mix thoroly by rolling, and place in a sample container. (Prepare the sample in an atmosphere of minimum moisture and $CO_2$ content and protect it from the breath of the operator.) Transfer a portion of the sample to a weighing bottle. By means of a polished, narrow-pointed spatula that has been calibrated to hold approximately 1.5 g, withdraw the charge to be used and determine its exact weight by difference. Introduce the charge directly into the dry flask (*A*), provided with a tightly fitting, rubber stopper.

Prepare a sugar soln *immediately before use* by placing 25 g of granulated sugar in a measuring flask calibrated to deliver 500 cc. Dissolve the sugar with cold $CO_2$-free $H_2O$ and make up to the mark. Hold both the Erlenmeyer flask containing the charge and the flask containing the sugar soln in a slightly inclined position, insert the neck of the sugar-soln flask a short distance into the Erlenmeyer flask, and carefully transfer the sugar soln while simultaneously and synchronously agitating both flasks by a rotary motion to prevent granulation of the lime. Stopper the Erlenmeyer flask securely; agitate; and add, if desired, a quantity of clean dry beads. Effect complete soln of uncoated caustic lime by six 1-min. agitations at intervals of 2 or 3 min. Crush any undisintegrated particles of the sample by careful twisting of the stopper after inverting the flask to trap them in the space between the stopper and the neck of the flask. Allow 15 min. further contact between the lime and the sugar soln, and then filter.

Connect the filter cone *F* with the syphon *B* and close stopcock *D*. Connect the receiving flasks, apply suction, and quickly connect the Erlenmeyer flask *A* containing the lime soln with stopper *E*. Open stopcock *C* and filter 25-50 cc of the soln. Close *C* and open *D* to release suction. Remove *m* and replace with another dry flask of the same kind. Close *D*, open *C*, and continue the filtration until both *m* and *n* have been filled at least to the marks. To disconnect the system, close stopcock *C* and press the outlet of flask *m* down gently and then the outlet of flask *n* to remove any excess of liquid above the marks. Permit the intermediate connection to empty, and then open stopcock *D* and remove *m* and *n*. Titrate the first 50 cc, or pilot aliquot, of the filtered soln with 0.5 *N* HCl, using phenolphthalein indicator. Run twice the volume of the 0.5 *N* acid required for this titration into a covered 200 cc beaker, add the second, or 100 cc aliquot, of the filtered soln to this acid and phenolphthalein indicator; and complete the titration.

Calculate the caustic value of the sample by means of the formula:

$$X=\frac{7A}{W}, \text{ in which}$$

$X$ = percentage of active CaO;
$A$ = cc of 0.5 $N$ acid used per 100 cc of lime soln;
$W$ = weight of charge.

### 7 CARBON DIOXIDE—TENTATIVE

Proceed as directed under **I, 6,** using 5 g of burnt or hydrated lime (1 g of ground limestone or ground marl), prepared as directed under **2**. Calculate and report the result as percentage of $CaCO_3$.

### 8 TOTAL CALCIUM OXIDE—TENTATIVE

Place 1 g of burnt or hydrated lime (2 g of ground limestone or ground marl), prepared as directed under **2**, in a hard glass beaker of 250 cc capacity; add 25 cc of $H_2O$, 10 cc of HCl, and a few drops of $HNO_3$; boil for 10 min. and evaporate to dryness. Separate and remove the insoluble matter, $SiO_2$, and Fe and Al oxides as directed under **I, 16** and **17**. Determine CaO as directed under **I, 18**.

### 9 TOTAL MAGNESIUM OXIDE—TENTATIVE

Proceed as directed under **I, 19** or **21**, using the combined filtrate and washings from the CaO determination under **8**.

### 10 MECHANICAL ANALYSIS OF GROUND LIMESTONE—TENTATIVE

Transfer 100 g of the original material to a set of 10-, 20-, 40-, 60-, 80-, and 100-mesh standardized sieves complying with the specifications of the Bureau of Standards. Sift, shaking for 5 min. on the 80- and 100-mesh sieves and breaking lumps by means of a soft rubber pestle if the material has a tendency to cake. Weigh the material that is retained on each sieve and that which passes the 100-mesh sieve and express as percentages of the total weight.

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[1] J. Assoc. Official Agr. Chem., 7, 252 (1924); U. S. Bur. Standards Circs., **96, 118, 143, 150, 153**; Am. Soc. Test. Materials, Tentative Standards, 1923, p. 277.
[2] Ind. Eng. Chem., **20**, 312 (1928); J. Assoc. Official Agr. Chem., **11**, 153 (1928.)

## V. AGRICULTURAL DUST*

* See note at bottom of p. xv.

# VI. INSECTICIDES AND FUNGICIDES

## GENERAL METHODS

### 1 PREPARATION OF SAMPLE—OFFICIAL

Thoroly mix all samples before analysis. Make water-soluble As determinations on samples as received, without further pulverization or drying. In the case of lye, NaCN, or KCN, weigh large quantities in weighing bottles and analyze aliquots of the aqueous solns.

### 2 MOISTURE—OFFICIAL

(Applicable to Paris green, London purple, powdered lead arsenate, calcium arsenate, magnesium arsenate, zinc arsenite, and powdered Bordeaux mixture.)

Dry 2 g to constant weight at 105-110° and report the loss in weight as moisture.

### TOTAL ARSENIC

#### *Method I (By Cuprous Chloride Distillation[1])—Official*

(Applicable except in the presence of nitrates to the determination of total arsenic in Paris green, lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

### 3 REAGENTS

(**a**) *Standard arsenious oxide soln.*—Dissolve 2 g of pure $As_2O_3$ in a beaker by boiling with about 150–200 cc of $H_2O$ containing 10 cc of $H_2SO_4$, cool, transfer to a 500 cc volumetric flask, and dilute to the mark.

(**b**) *Standard iodine soln.*—Prepare an approximately 0.05 *N* soln as follows: Mix intimately 6.35 g of pure I with twice this weight of pure KI. Dissolve in a small quantitiy of water, filter, and dilute the filtrate to 1 liter in a volumetric flask. Standardize against (**a**) as follows: Pipet 50 cc of the $As_2O_3$ soln into an Erlenmeyer flask; dilute to the same volume as that of the aliquot used for the titration in the actual determination; neutralize with $NaHCO_3$; add 4-5 g in excess; and add the standard I soln from a buret, shaking the flask continuously until the yellow color disappears slowly from the soln. Then add 5 cc of the starch indicator and continue adding the I soln, dropwise, until a permanent blue color is obtained. Calculate the value of the standard I soln in terms of $As_2O_3$ and $As_2O_5$. For the conversion of $As_2O_3$ to $As_2O_5$ multiply by 1.1617. Occasionally restandardize the I against the standard $As_2O_3$ soln.

(**c**) *Standard bromate soln.*—Dissolve 1.525 g of $NaBrO_3$ in $H_2O$ and dilute to 1 liter. One cc of this soln is approximately equal to 0.003 g of $As_2O_3$. To standardize, transfer 25 cc aliquots of the standard $As_2O_3$ soln (**a**) to 500 cc Erlenmeyer flasks; add 15 cc of HCl; dilute to 100 cc; heat to 90°; and titrate with the bromate soln, using 5 drops of the methyl orange indicator (**f**). Do not add the indicator until near the end of the titration, and agitate the liquid continuously in order to avoid local excess of the bromate soln. Add the bromate soln very slowly when approaching the end of the titration; the end point is shown by a change from red to colorless.

(**d**) *Sodium hydroxide soln.*—Dissolve 400 g of NaOH in $H_2O$ and dilute to 1 liter.

(**e**) *Starch indicator.*—Mix about 2 g of finely powdered potato starch with cold $H_2O$ to a thin paste; add about 200 cc of boiling $H_2O$, stirring constantly; and immediately discontinue heating.

(f) *Methyl orange indicator.*—Dissolve 1 g of methyl orange in $H_2O$ and dilute to 1 liter.

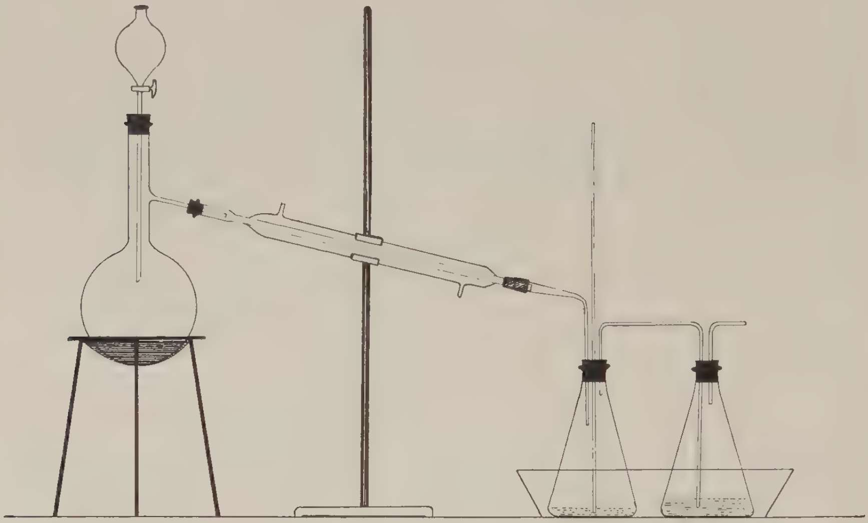


FIG. 4.—APPARATUS FOR DISTILLATION OF ARSENIOUS CHLORIDE

4

### APPARATUS

The apparatus used is shown in Fig. 4. The distillation flask is of 500 cc capacity and rests on a metal gauze that fits over a circular hole in a heavy sheet of asbestos board, which in turn extends out far enough to protect the sides of the flask from the direct flame of the burner. The first receiving flask is of 500 cc capacity and contains 40 cc of $H_2O$; the second is also of 500 cc capacity and contains 100 cc of $H_2O$. The volume in the first flask should not exceed 40 cc, otherwise there may be separated a compound of As that can not readily be redissolved without danger of loss of $AsCl_3$. Keep both flasks cool by placing them in a pan thru which $H_2O$ circulates, or which contains $H_2O$ and pieces of ice.

5

### DETERMINATION

Weigh a quantity of the sample containing not more than 0.4 g of As and wash into the distillation flask by means of 100 cc of HCl. Add 5 g of $Cu_2Cl_2$ and distil. When the volume in the distillation flask is reduced to about 40 cc add 50 cc more of HCl by means of the dropping funnel and continue the distillation, repeating the additions of 50 cc portions of HCl until 200 cc of the acid distillate has passed over. Wash down the condenser and all connecting tubes carefully, transfer these washings and the contents of the Erlenmeyer flasks to a liter volumetric flask, dilute to the mark, and mix thoroly. Titrate the distillate by one of the following procedures:

(a) Pipet a 200 cc aliquot into an Erlenmeyer flask and nearly neutralize with the NaOH soln, using a few drops of phenolphthalein indicator [**II, 8**(**d**)] and keeping the soln well cooled. If the neutral point is passed, add HCl until again slightly acid. Neutralize with $NaHCO_3$; add 4–5 g in excess; and add the standard I soln from a buret, shaking the flask continuously until the yellow color disappears slowly from the soln. Then add 5 cc of the starch indicator and continue adding the I soln dropwise until a permanent blue color is obtained.

**(b)**[2] Pipet a 200 cc aliquot into an Erlenmeyer flask and titrate with the standard bromate soln as directed under **3(c)**, beginning with "heat to 90°."

From the number of cc of standard soln used, calculate the percentage of As in the sample. Report as $As_2O_3$ or $As_2O_5$, according to whether the As is present in the trivalent or pentavalent form. If the condition of the arsenic is unkown, report as As.

### *Method II (By Hydrazine Sulfate Distillation[3])—Official*

(Nitrates do not interfere in this method. Applicable to the determination of total arsenic in Paris green, lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

#### **6** REAGENTS

*Hydrazine sulfate-sodium bromide soln.*—Dissolve 20 g of hydrazine sulfate and 20 g of NaBr in 1 liter of HCl (1+4).

The other reagents and solns used are described under **3.**

#### **7** APPARATUS

The apparatus and manner of connecting are described under **4** (Fig. 4).

#### **8** DETERMINATION

Weigh a quantity of the sample containing not more than 0.4 g of As and transfer to the distilling flask. Add 50 cc of the hydrazine sulfate-NaBr soln, close the flask with the stopper that carries the funnel tube, and connect the side tube with the condenser. Boil for 2–3 min., add 100 cc of HCl by means of the dropping funnel, and distil until the volume in the distilling flask is reduced to about 40 cc; add 50 cc more of HCl and continue the distillation until the contents of the flask are again reduced to about 40 cc. Wash down the condenser, transfer the contents of the receiving flask to a liter volumetric flask, dilute to volume, and mix thoroly. Titrate the distillate by one of the following procedures:

**(a)** Proceed as directed under **5(a)**; or

**(b)** Pipet a 200 cc aliquot into an Erlenmeyer flask, add 10 cc of HCl, and titrate with the standard bromate soln as described under **3(c)**, beginning with "heat to 90°."

From the number of cc of standard soln used, calculate the percentage of As in the sample. Report as $As_2O_3$ or $As_2O_5$, according to whether the As is present in the trivalent or pentavalent form. If the condition of the arsenic is unknown, report as As.

### *Method III[4]—Tentative*

(Applicable in presence of sulfides, sulfites, thiosulfates or large quantities of sulfur.)

#### **9** REAGENTS

*Sodium thiosulfate soln.*—Prepare an approximately 0.05 *N* soln by dissolving 13 g of crystallized $Na_2S_2O_3 . 5H_2O$ in $H_2O$ and diluting to 1 liter.

The other reagents and solns used are described under **3.**

### 10 APPARATUS

The apparatus and manner of connecting are described under **4** (Fig. 4).

### 11 DETERMINATION

Weigh 2 g of the sample and transfer to the distilling flask. Add a soln of 5–8 g of $Cu_2Cl_2$ in 100 cc of HCl and shake to bring the sample completely in contact with the acid soln and to expel $H_2S$. When the reaction has ceased, close the flask, connect with the condenser, and distil as directed under 5 until 200 cc of the acid distillate has passed over. Make the distillate to volume in a liter flask, mix thoroly, and transfer a 200 cc aliquot to a 400 cc Pyrex beaker or porcelain casserole. Add 10 cc of $HNO_3$ and 5 cc of $H_2SO_4$, evaporate to a sirupy consistency on a steam bath, and then heat on a hot plate until the white fumes of $H_2SO_4$ appear. Cool, and wash into a 500 cc Erlenmeyer flask. If the quantity of $H_2SO_4$ is appreciably lessened by fuming, add sufficient to make the total quantity of $H_2SO_4$ approximately 5 cc. Dilute to 100–150 cc, add 1.5 g of KI, and boil until the volume is reduced to about 40 cc. Cool the soln under running $H_2O$; dilute to 100–150 cc; and add the $Na_2S_2O_3$ soln, **9**, dropwise until the I color is just removed. Nearly neutralize the $H_2SO_4$ with the NaOH soln, **3(d)**, finish the neutralization with $NaHCO_3$, add 4–5 g in excess, and titrate with the standard I soln as directed under **3(b)**. From the number of cc of standard soln used calculate the percentage of As in the sample. Report as $As_2O_3$ or $As_2O_5$ according to whether the As is present in the trivalent or pentavalent form. If the condition of the arsenic is unknown, report as As.

## WATER-SOLUBLE ARSENIC—OFFICIAL

(Applicable to the determination of water-soluble arsenic in lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

### 12 REAGENTS

The reagents and solns used are described under **3** and **9**.

### 13 DETERMINATION

To 2 g of the original sample if a powder, or 4 g if a paste, in a liter Florence flask, add 1 liter of recently boiled $H_2O$ that has been cooled to 32°. Stopper the flask and place in a water bath kept at 32° by means of a thermostat. Digest for 24 hours, shaking hourly for 8 hours during this period. Filter thru a dry filter, transfer 250–500 cc of the *clear* filtrate to an Erlenmeyer flask, add 3 cc of $H_2SO_4$, and evaporate on a hot plate. When the volume reaches approximately 100 cc add 1 g of KI, and continue the boiling until the volume is about 40 cc. Cool, dilute to about 200 cc, and add the $Na_2S_2O_3$ soln, **9**, dropwise until the I color is exactly removed. (Avoid the use of starch indicator at this point.) Neutralize with $NaHCO_3$, add 4–5 g in excess, titrate with the standard I soln until the yellow color disappears slowly, add 5 cc of the starch indicator, and continue the titration to a permanent blue color. Make correction for the quantity of standard I soln necessary to produce the same color, using the same reagents and volume. From the number of cc of standard I soln used calculate the percentage of water-soluble As in the sample.

## GENERAL PROCEDURE FOR THE ANALYSIS OF PRODUCTS CONTAINING ARSENIC, ANTIMONY, LEAD, COPPER, ZINC, IRON, CALCIUM, MAGNESIUM, ETC.

(Applicable to such preparations as Bordeaux-lead arsenate, Bordeaux-zinc arsenite, Bordeaux-Paris green, and Bordeaux-calcium arsenate.)

## LEAD OXIDE[5]—OFFICIAL

### 14 REAGENTS

(**a**) *Acidified alcohol.*—Mix by volume $H_2O$, 100 parts; 95% alcohol, 200 parts; and $H_2SO_4$, 3 parts.

(**b**) *Hydrochloric acid.*—Approximately 2 *N*. Dilute 166 cc of HCl to 1 liter.

(**c**) *Hydrochloric acid.*—Approximately 0.5 *N*. Dilute 250 cc of (**b**) to 1 liter.

### 15 DETERMINATION

Weigh 1 g of the powdered sample and transfer to a beaker. Add 5 cc of HBr (approximately 1.38 sp. gr.) and 15 cc of HCl, and evaporate to dryness to remove As; repeat the treatment; add 20 cc more of the HCl; and again evaporate to dryness. Add to the residue 25 cc of the 2 *N* HCl, heat to boiling, filter immediately to remove $SiO_2$, and wash with hot $H_2O$ to a volume of 125 cc. Take care to see that all $PbCl_2$ is in soln before filtering; if it will not dissolve completely in 25 cc of the 2 *N* acid, add 25 cc additional and dilute the filtrate to 250 cc volume. Pass in $H_2S$ until the precipitation is complete. Filter, and wash the precipitate thoroly with the 0.5 *N* HCl saturated with $H_2S$. Save the filtrate and washings for the determination of zinc. Transfer the filter paper containing the sulfides of Pb and Cu to a 400 cc Pyrex beaker and completely oxidize all organic matter by heating on a steam bath with 4 cc of $H_2SO_4$ and about 20 cc of fuming $HNO_3$ in a covered beaker; evaporate on the steam bath and then completely remove $HNO_3$ by heating on a hot plate until copious evolution of the white fumes of $H_2SO_4$ occurs, cool, add 2–3 cc of $H_2O$, and again heat to fuming. Cool, add 50 cc of $H_2O$ and 100 cc of 95% alcohol, and let stand several hours (preferably overnight). Filter thru a Gooch crucible, previously washed with $H_2O$, with the acidified alcohol, and with 95% alcohol, and then dried at 200°. Wash the precipitate of $PbSO_4$ in the crucible about 10 times with the acidified alcohol, and then with 95% alcohol, to remove $H_2SO_4$. Dry at 200° to constant weight, keeping the crucible covered to prevent loss from spattering. From the weight of $PbSO_4$ calculate the percentage of PbO in the sample, using the factor 0.7360.

## COPPER[5]

### 16 *Electrolytic Method—Official*

Evaporate the filtrate and washings from the $PbSO_4$ precipitation, **15**, to fuming; add a few cc of fuming $HNO_3$ to destroy organic matter; and continue the evaporation until about 3 cc remains. Take up with about 100 cc of $H_2O$, add 1 cc of HNO, and filter, if necessary. Wash into a weighed 150 cc Pt dish and electrolyze, using a rotating anode and a current of about 3 amperes. (In lieu of the Pt dish a 150 cc beaker and a weighed gauze cathode may be used.) After all the Cu has been deposited (requiring about 30 min.) and while the current is still flowing, wash the deposit with $H_2O$ by siphoning. Then interrupt the current, rinse the cathode with alcohol, dry for a few moments in an oven, and weigh. Calculate the percentage of Cu in the sample.

### 17 *Thiosulfate Titration Method—Official*

Proceed as directed under **16** to the point at which the filtrate and washings from the $PbSO_4$ precipitation have been treated with fuming $HNO_3$ and evaporated to a volume of about 3 cc. Take up in about 50 cc of $H_2O$, add $NH_4OH$ in excess, and boil until the excess of $NH_3$ is expelled, as shown by a change of color in the liquid and a partial precipitation. Then add 3–4 cc of acetic acid (80%), boil 1–2 min., cool, add 10 cc of a 30% KI soln, and titrate with standard thiosulfate soln

(**XXXIV, 40**) until the brown color becomes faint. Then add starch indicator, **3**(**e**), and continue the titration cautiously until the blue color due to free I has entirely vanished. From the number of cc of standard thiosulfate soln used calculate the percentage of Cu in the sample.

### ZINC OXIDE[6]—OFFICIAL

**18** REAGENT

*Mercury-thiocyanate soln.*—Dissolve 27 g of $HgCl_2$ and 30 g of $NH_4SCN$ in $H_2O$ and dilute to 1 liter.

**19** DETERMINATION

Concentrate the filtrate and washings from the sulfide precipitation, under **15**, by gentle boiling to about 50 cc, and continue the evaporation on a steam bath to dryness. Dissolve the residue in 100 cc of $H_2O$ containing 5 cc of HCl, and add 35–40 cc of the Hg-thiocyanate reagent with vigorous stirring. Allow to stand at least an hour with occasional stirring. Filter thru a weighed Gooch crucible, wash with $H_2O$ containing 20 cc of the Hg-thiocyanate reagent per liter, and dry to constant weight at 105°. From this weight calculate the percentage of ZnO in the sample, using the factor 0.16332.

Some Fe is usually present and during the Zn determination should be in the ferrous condition. In making the sulfide precipitation the $H_2S$ should be passed into the soln for a sufficient length of time to reduce the Fe, as well as to precipitate the Cu and Pb. The Zn-Hg-thiocyanate precipitate normally is white, and the occluded ferric thiocyanate should not give it more than a faint pink color.

## PARIS GREEN

**20** MOISTURE—OFFICIAL

Proceed as directed under **2**.

**21** TOTAL ARSENIC—OFFICIAL

Proceed as directed under **5** or **8**.

### TOTAL ARSENIOUS OXIDE

(The following methods determine only the As present in the trivalent form ($As_2O_3$). They also determine any Sb that may be present in the trivalent form ($Sb_2O_3$). Ferrous and cuprous salts vitiate the results.)

*Method I[7]—Official*

**22** REAGENTS

*Ammonium chloride soln.*—Dissolve 250 g of $NH_4Cl$ in $H_2O$ and dilute to 1 liter. The other reagents and solns are described under **3**.

**23** DETERMINATION

Weigh 0.3 g of the sample and wash into an Erlenmeyer flask with 10–15 cc of HCl (1+4) or 10–15 cc of $H_2SO_4$ (1+4), followed by about 100 cc of $H_2O$, and heat on a steam bath only so long as is necessary to complete soln, at a temp. not exceeding 90°. (If $H_2SO_4$ is used the soln may be heated to boiling.) Cool, neutralize with $NaHCO_3$, add 4–5 g in excess, and then add sufficient $NH_4Cl$ soln to dissolve the precipitated Cu. Dilute somewhat and titrate as directed under **3**(**b**). Make correction for the quantity of I soln necessary to produce a blue color with starch in the presence of Cu (using an equivalent weight of $CuSO_4$). From the corrected number of cc of standard I soln used calculate the percentage of $As_2O_3$ in the sample.

### *Method II*[8]—*Official*

**24** REAGENTS

The reagents and solns used are described under **3**.

**25** DETERMINATION

Weigh 1.5 g of the sample and wash into a 250 cc volumetric flask with 100 cc of HCl (1+4), heating to a maximum of 90°, if necessary, to secure complete soln of the sample. Cool, and make to volume.

(**a**) Transfer a 50 cc aliquot to a 500 cc Erlenmeyer flask, add 10 cc of HCl, heat to 90°, and titrate with the standard bromate soln as directed under **3**(**c**), beginning with "titrate with the bromate soln." Or,

(**b**) Proceed as directed under (**a**) with the exception that the titration is made without heating the soln.

From the number of cc of bromate soln used calculate the percentage of $As_2O_3$ in the sample.

## SODIUM ACETATE-SOLUBLE ARSENIOUS OXIDE[9]—TENTATIVE

**26** REAGENTS

(**a**) *Sodium acetate soln.*—Prepare a soln containing 12.5 g of the crystallized salt ($CH_3COONa.3H_2O$) in each 25 cc.

The other reagents and solns used are described under **3**.

**27** DETERMINATION

Place 1 g of the sample in a 100 cc volumetric flask and boil for 5 min. with 25 cc of the Na-acetate soln. Dilute to the mark, shake, and pass thru a dry filter paper. Titrate an aliquot of this filtrate as directed under **3**(**b**). Calculate the quantity of $As_2O_3$ present and express the results as percentage of Na-acetate-soluble $As_2O_3$.

## WATER-SOLUBLE ARSENIOUS OXIDE—OFFICIAL

**28** REAGENTS

The reagents and solns used are described under **3**.

**29** DETERMINATION

To 1 g of the sample in a liter Florence flask add 1 liter of recently boiled $H_2O$ that has been cooled to 32°. Stopper the flask and place in a water bath kept at 32° by means of a thermostat. Digest for 24 hours, shaking hourly for 8 hours during this period. Filter thru a dry filter and transfer 250 cc of the filtrate to an Erlenmeyer flask; add 4–5 g of $NaHCO_3$; and titrate with the standard I soln to a permanent blue color, using starch indicator. Correct for the quantity of the standard I necessary to produce the same color, using the same reagents and volume. Calculate the quantity of $As_2O_3$ present and express the results as percentage of water-soluble $As_2O_3$.

## TOTAL COPPER OXIDE

**30** *Electrolytic Method—Official*

Treat 2 g of the sample in a beaker with 100 cc of $H_2O$ and about 2 g of NaOH and boil thoroly until all the Cu is precipitated as $Cu_2O$. Filter, wash well with hot

$H_2O$, dissolve the precipitate in hot $HNO_3$ (1+4), cool, transfer to a 250 cc volumetric flask, and dilute to the mark. Electrolyze an aliquot of 50 or 100 cc, as directed under **16.** Calculate to percentage of CuO.

### **31** *Thiosulfate Method*[10]*—Official*

Determine Cu in an aliquot of the $HNO_3$ soln of $Cu_2O$, under **30,** by titrating with standard thiosulfate soln, as directed under **17,** and calculate to percentage of CuO.

## LONDON PURPLE

### **32** MOISTURE—OFFICIAL

Proceed as directed under **2.**

### TOTAL ARSENIC

### *Zinc Oxide-Sodium Carbonate Method*[11]*—Official*

### **33** REAGENTS

(**a**) *Zinc oxide-sodium carbonate mixture.*—Mix 4 parts of ZnO and 1 part of anhydrous $Na_2CO_3$.

The other reagents and solns used are described under **3.**

### **34** DETERMINATION

Mix 1 g of the sample thoroly with several times its weight of the ZnO-$Na_2CO_3$ reagent in a shallow porcelain crucible, and cover with a layer of the same reagent. Place the crucible, uncovered, in a muffle and heat, gently at first and finally for about 15 min. at full heat. (The mass will not sinter.) Cool, transfer to a 500 cc distillation flask and proceed as directed under **5** or **8.**

### TOTAL ARSENIOUS OXIDE[12]—OFFICIAL

### **35** REAGENTS

The reagents and solns used are described under **3.**

### **36** DETERMINATION

Dissolve 2 g of the sample in 100 cc of HCl (1+4) at a temp. not exceeding 90°, filter into a 250 cc volumetric flask, wash thoroly, cool, and dilute to volume. Treat 100 cc of this soln with $NaHCO_3$ in excess; transfer to a 500 cc volumetric flask; dilute to the mark, adding a few drops of ether to destroy the bubbles. Mix thoroly and pass thru a dry filter. Titrate 250 cc of the filtrate as directed under **3**(**b**) and calculate the percentage of $As_2O_3$.

### TOTAL ARSENIC OXIDE[13]—OFFICIAL

### **37** REAGENTS

The reagents and solns used are described under **3.**

### **38** DETERMINATION

Boil on a hot plate or over a low flame 2 g of the sample with 5 cc of $HNO_3$ and 20 cc of $H_2SO_4$ in a covered casserole or a Kjeldahl digestion flask. After 10–15 min. add fuming $HNO_3$ or powdered $NaNO_3$, in small quantities at a time, until all organic matter is destroyed and the soln is practically colorless. Cool, add about 50 cc of $H_2O$ (to decompose any nitro-sulfuric acid formed), and heat again until all $HNO_3$ fumes are expelled. Cool, transfer to a 250 cc volumetric flask, dilute to the mark with $H_2O$, mix thoroly, and filter thru a dry filter.

Transfer 50 cc of this filtrate to a 400 cc Erlenmeyer flask, dilute with $H_2O$ to 100 cc, add 1 g of KI, heat to boiling, and evaporate to about 40 cc (not less). Cool, dilute to 150–200 cc, and remove the excess of I with 0.05 *N* $Na_2S_2O_3$, **9.** If the soln is slightly colored from organic matter or from any materials other than free I add the thiosulfate until it is nearly colorless, then a few drops of the starch indicator, and continue adding the thiosulfate slowly until the blue color just disappears. Continue at once as directed under **3(b)**, beginning with "neutralize with $NaHCO_3$." Subtract from this reading the number of cc of the standard I soln corresponding to the $As_2O_3$ obtained under **36** and from the remainder calculate the percentage of $As_2O_5$ in the sample.

### 39 WATER-SOLUBLE ARSENIOUS OXIDE—OFFICIAL

Proceed as directed under **29**, using 2 g of the sample and slightly acidifying the aliquot employed with HCl (1+4) before adding the excess of $NaHCO_3$.

### WATER-SOLUBLE ARSENIC OXIDE—OFFICIAL

#### 40 REAGENTS

The reagents and solns used are described under **3.**

#### 41 DETERMINATION

Transfer a 250 cc aliquot of the $H_2O$ extract obtained under **39** to a casserole, add 5 cc of $H_2SO_4$, evaporate to a small volume, and heat on a hot plate until white fumes of $H_2SO_4$ appear. Cover the casserole, add 1–2 cc of fuming $HNO_3$, and again heat until the appearance of white fumes. Cool, add a little $H_2O$, and, in order to expel the last traces of $HNO_3$, once more evaporate until white fumes appear. Cool, dilute to about 100 cc with $H_2O$, and add 1 g of KI and sufficient $H_2SO_4$ to make the total quantity present about 5 cc. Boil until the volume is reduced to about 40 cc. Cool, dilute to about 200 cc, and add the approximately 0.05 *N* $Na_2S_2O_3$ soln, **9,** dropwise until the I color is exactly removed. Proceed as directed under **3(b)**, beginning with "neutralize with $NaHCO_3$." Correct for the quantity of the standard I soln necessary to produce the same color, using the same reagents and volume. Subtract from the corrected titration reading the number of cc of the standard I soln corresponding to the $As_2O_3$ obtained under **39**, and from the remainder calculate the percentage of $As_2O_5$ present.

## LEAD ARSENATE

### 42 MOISTURE—OFFICIAL

**(a)** *Powder.*—Dry 2 g to constant weight at 105–110° and report the loss in weight as moisture.

**(b)** *Paste.*—Proceed as directed under (a), using 50 g. Grind the dry sample to a fine powder, mix well, transfer a small portion to a sample bottle, and again dry for 1–2 hours at 105–110°. Use this anhydrous material for the determination of total PbO and total As.

### TOTAL ARSENIC

#### 43 *Method I—Official*

Proceed as directed under **5** or **8.**

### *Method II*[14]*—Official*

(Not applicable in the presence of antimony)

**44** REAGENTS

The reagents and solns used are described under **3.**

**45** DETERMINATION

Dissolve 1 g of the powdered sample in $HNO_3$ (1+4) in a porcelain casserole or evaporating dish, add 5 cc of $H_2SO_4$ and heat on a hot plate to copious evolution of white fumes. Cool, add a little $H_2O$, and again evaporate until the appearance of white fumes to assure removal of the last trace of $HNO_3$. Wash into a 200 cc volumetric flask with $H_2O$, cool, dilute to volume, and filter thru a dry filter. Transfer 100 cc of the filtrate to an Erlenmeyer flask and proceed as directed under **13,** beginning with "add 1 gram of KI." From the number of cc of standard I soln used calculate the percentage of total As in the sample in terms of $As_2O_5$.

## TOTAL ARSENIOUS OXIDE[15]—OFFICIAL

**46** REAGENTS

The reagents and solns used are described under **3.**

**47** DETERMINATION

Weigh 2 g of the powdered sample and transfer to a 200 cc volumetric flask, add 100 cc of $H_2SO_4$ (1+6), and boil for 30 min. Cool, dilute to volume, shake thoroly, and filter thru a dry filter. Nearly neutralize 100 cc of the filtrate with NaOH soln, **3(d)**, using a few drops of phenolphthalein indicator. If the neutral point is passed, make acid again with the dilute $H_2SO_4$. Continue as directed under **3(b)**, beginning "neutralize with $NaHCO_3$." From the number of cc of standard I soln used calculate the percentage of $As_2O_3$.

## TOTAL ARSENIC OXIDE[16]—TENTATIVE

**48** REAGENTS

**(a)** *Starch indicator.*—Prepare as directed under **3(e)**.

**(b)** *Ammonium chloride soln.*—Dissolve 250 g of $NH_4Cl$ in $H_2O$ and dilute to 1 liter.

**(c)** *Potassium iodide soln.*—Dissolve 20 g of KI in $H_2O$ and dilute to 100 cc.

**(d)** *Standard iodine soln.*—Prepare as directed under **3(b)**, but calculate in terms of $As_2O_5$.

**(e)** *Standard thiosulfate soln.*—Prepare an approximately 0.05 *N* soln as follows: Dissolve 13 g of $Na_2S_2O_3.5H_2O$ in recently boiled and cooled $H_2O$, filter, and dilute to 1 liter with recently boiled and cooled $H_2O$. Standardize as follows:

Dissolve about 0.7 g of $PbHAsO_4$ in 50 cc of HCl in an Erlenmeyer flask. If necessary to effect soln, heat on a steam bath, keeping the flask covered with a watch-glass to prevent evaporation of the acid. Cool to 20–25°, add 10 cc of the KI soln **(c)** and 50 cc (or more if necessary to produce a clear soln) of the $NH_4Cl$ soln, **(b)**, and immediately titrate the liberated I with the standard thiosulfate. When the color becomes a faint yellow, dilute with about 150 cc of $H_2O$ and continue the titration carefully, dropwise, until colorless, using the starch indicator near the end point. From the weight of $PbHAsO_4$ and the number of cc of $Na_2S_2O_3$ soln used calculate the value of the latter in terms of $As_2O_5$. ($As_2O_5$ in $PbHAsO_4$ = 33.11 per cent.)

Pure $PbHAsO_4$ may be prepared by pouring a soln of $Pb(NO_3)_2$ into a soln of $KH_2AsO_4$, which should be in excess. Collect the precipitate by filtration, dissolve it in the smallest possible quantity of boiling $HNO_3$ (1+4), and pour the soln into a large quantity of $H_2O$. Collect the precipitate by filtration and dry at 110°.

### 49 DETERMINATION

Weigh 0.5 g of the powdered sample and transfer to an Erlenmeyer flask. Add 25–30 cc of HCl and evaporate to dryness on a steam bath. Then add 50 cc of HCl and proceed as directed under **48**(e), beginning with "If necessary to effect soln, heat on a steam bath." From the number of cc of standard thiosulfate soln used calculate the percentage of $As_2O_5$.

### 50 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under **13**, and calculate results as $As_2O_5$.

## TOTAL LEAD OXIDE

### 51 *Method I*[17]*—Official*

Heat, on a hot plate, 0.5 g of the powdered sample with about 25 cc of $HNO_3$ (1+4) in a 600 cc beaker. Remove any insoluble residue by filtration. Dilute to at least 400 cc; heat nearly to boiling; and add $NH_4OH$ to slight precipitation, then $HNO_3$ (1+9) to redissolve the precipitate, adding 1–2 cc in excess. Pipet into this soln, kept almost boiling, 50 cc of a hot 10% $K_2CrO_4$ soln, stirring constantly. Decant while hot thru a weighed Gooch crucible, previously heated to 140–150°, and wash several times by decantation and then on the filter with boiling $H_2O$ until the washings are colorless. Dry the $PbCrO_4$ at 140–150° to constant weight. From the weight of the $PbCrO_4$ calculate the percentage of PbO in the sample, using the factor 0.6906

The $PbCrO_4$ precipitate may contain a small quantitiy of $PbHAsO_4$, which will cause slightly high results, but this error rarely amounts to more than 0.1–0.2%.

### 52 *Method II*[18]*—Official*

(Not applicable in the presence of calcium)

Heat, on a hot plate, 0.5 g of the powdered sample with about 25 cc of $HNO_3$ (1+4) in a porcelain evaporating dish or casserole. Remove any insoluble residue by filtration. Add 3 cc of $H_2SO_4$ and evaporate on a hot plate until the appearance of white fumes. Cool, add a few cc of $H_2O$ (to decompose any nitro-sulfuric acid formed), and again heat to fuming. Proceed as directed under **15**, beginning with "Cool, add 50 cc of $H_2O$ and 100 cc of 95% alcohol."

## CALCIUM ARSENATE

### 53 MOISTURE—OFFICIAL

Proceed as directed under **2**.

### 54 TOTAL ARSENIC

Proceed as directed under **5** or **8**.

## TOTAL ARSENIOUS OXIDE[19]—OFFICIAL

### 55 REAGENTS

The reagents and solns used are described under **3**.

### 56 DETERMINATION

**(a)** (Not applicable in the presence of nitrates.)

Weigh 1 g of the sample, transfer to a 500 cc Erlenmeyer flask, and dissolve in 100 cc of HCl (1+3). Heat to 90° and titrate with the standard bromate soln, using 5 drops of the methyl orange indicator. From the number of cc of standard bromate soln used calculate the percentage of $As_2O_3$ in the sample.

**(b)** (Applicable in the presence of small quantities of nitrates.)

Proceed as directed under **(a)** except to make the titration at room temp.

### 57 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under **13**, and calculate results as $As_2O_5$.

## TOTAL CALCIUM OXIDE

### *Method I*[19]*—Official*

### 58 REAGENTS

**(a)** *Ammonium oxalate soln.*—Dissolve 40 g of $(NH_4)_2C_2O_4 \cdot H_2O$ in 1 liter of $H_2O$.

**(b)** *Standard potassium permanganate soln.*—Dissolve 3.161 g of $KMnO_4$ in freshly distilled $H_2O$ and dilute to 1 liter. Filter thru asbestos in a Gooch crucible and allow to stand for several days in a dark place. To standardize, dissolve 0.25 g of pure $Na_2C_2O_4$ in water, add 25 cc of $H_2SO_4$ (1+4), dilute to 200 cc, heat to about 70°, and titrate with the $KMnO_4$ soln until the soln assumes a faint pink color. From this titration calculate the strength of the $KMnO_4$ soln, which should be approximately 0.1 *N*.

### 59 DETERMINATION

Dissolve 2 g of the sample in 80 cc of acetic acid (1+3), transfer to a 200 cc volumetric flask, and dilute to volume. Filter thru a dry filter and transfer a 50 cc aliquot to a beaker; dilute to about 200 cc; heat to boiling; and precipitate the Ca with the $(NH_4)_2C_2O_4$ soln. Allow the beaker to stand 3 hours on a steam bath, filter, and wash with hot $H_2O$. Dissolve the precipitate in 200 cc of $H_2O$ containing 25 cc of $H_2SO_4$ (1+4), heat to about 70°, and titrate with the standard $KMnO_4$ soln. From the number of cc of standard $KMnO_4$ soln used calculate the percentage of CaO.

### *Method II*[19]*—Official*

(Not applicable in the presence of lead)

### 60 REAGENTS

The reagents and solns used are described under **58**.

### 61 DETERMINATION

Weigh 2 g of the sample; transfer to a beaker; add 5 cc of HBr (approximately 1.38 sp. gr.) and 15 cc of HCl; and evaporate to dryness, under a hood, to remove As. Repeat the treatment, add 20 cc of HCl, and again evaporate to dryness. Take up with $H_2O$ and a little HCl, filter into a 200 cc volumetric flask, wash, and dilute to volume. Transfer a 50 cc aliquot to a beaker; add 10 cc of HCl and a few drops of $HNO_3$; boil; and make slightly alkaline with $NH_4OH$. Let stand a few min. and filter. Dissolve the precipitate in HCl (1+4), reprecipitate, filter thru the same paper, and wash with hot $H_2O$. To the combined filtrates and washings add 20 cc of acetic acid (1+3) and adjust the volume to about 200 cc. Heat to boiling, precipitate with the $(NH_4)_2C_2O_4$ soln, and allow to stand for 3 hours on a steam bath. Filter and wash with hot $H_2O$. Ignite, and weigh as CaO; or dissolve the precipitate in 200 cc of $H_2O$ containing 25 cc of $H_2SO_4$ (1+4), heat to about 70°, and titrate with the standard $KMnO_4$ soln. From the weight of CaO or the number of cc of standard $KMnO_4$ soln used calculate the percentage of CaO.

## MAGNESIUM ARSENATE

### 62 MOISTURE—OFFICIAL

Proceed as directed under **2**.

### 63 TOTAL ARSENIC—OFFICIAL

Proceed as directed under **5** or **8**.

### 64 TOTAL ARSENIOUS OXIDE—OFFICIAL

Proceed as directed under **47**.

### 65 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under **13**, and calculate the results as $As_2O_5$.

## ZINC ARSENITE

### 66 MOISTURE—OFFICIAL

Proceed as directed under **2**.

### 67 TOTAL ARSENIC—OFFICIAL

Proceed as directed under **5** or **8**, and calculate results as $As_2O_3$.

### TOTAL ARSENIOUS OXIDE

*Method I*[19]*—Official*

### 68 REAGENTS

The reagents and solns used are described under **3**.

### 69 DETERMINATION

(**a**) Weigh 2 g of the sample and transfer to a beaker. Dissolve in 80 cc of HCl (1+4), wash into a 200 cc volumetric flask, and dilute to volume. Thoroly mix the soln and filter thru a dry filter. Transfer a 25 cc aliquot to a 500 cc Erlenmeyer flask, add 20 cc of HCl, and dilute to 100 cc. Heat to 90° and titrate with the standard bromate soln. Or,

(**b**) Proceed as directed under (**a**) without heating the soln.

### 70 *Method II—Official*

Proceed as directed under **23**.

### 71 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under **13**, and calculate results as $As_2O_3$.

### TOTAL ZINC OXIDE[19]—OFFICIAL

### 72 REAGENT

The reagent used is described under **18**.

### 73 DETERMINATION

Transfer a 25 cc aliquot of the soln prepared for the determination of total $As_2O_3$, **69**, to a beaker and add 5 cc of HCl. If there is much Fe present, reduce it at this point by adding a little $NaHSO_3$ and heating on a steam bath until the odor of $SO_2$ has largely disappeared. Cool, dilute to about 100 cc, and proceed as directed under **19**, beginning with "add 35–40 cc of the Hg-thiocyanate reagent with vigorous stirring."

## COPPER CARBONATE

### COPPER OXIDE

**74** *Electrolytic Method—Official*

Weigh 0.5 g of the sample, transfer to a 150 cc Pt dish or 150 cc beaker, and dissolve in 25 cc of $HNO_3$ (1+4). Dilute to about 100 cc and determine the Cu by electrolysis, as directed under **16**, beginning with "electrolyze, using a rotating anode and a current of about 3 amperes."

**75** *Thiosulfate Method—Official*

Dissolve 0.25–0.5 g of the sample in 25 cc of $HNO_3$ (1+4), dilute to about 50 cc, and proceed as directed under **17**, beginning with "add $NH_4OH$ in excess."

## BORDEAUX MIXTURE

**76**

### MOISTURE—OFFICIAL

(**a**) *Powder.*—Dry 2 g to constant weight at 105–110° and report the loss as moisture.

(**b**) *Paste.*—Heat about 100 g in an oven at 90–100° until dry enough to powder readily and note the loss in weight. Powder this partially dried sample and determine the remaining moisture in 2 g, as directed under (**a**). Determine $CO_2$, as directed under **78**, both in the original paste and in this partially dried sample. Calculate the total moisture by the following formula:

$$M = a + \frac{(100-a)\,(b+c)}{100} - d, \text{ in which}$$

$M$ = percentage of total moisture in original paste;
$a$ = percentage of loss in weight of original paste during first drying;
$b$ = percentage of loss in weight of partially dried paste during second drying;
$c$ = percentage of $CO_2$ remaining in partially dried paste after first drying; and
$d$ = percentage of total $CO_2$ in original paste.

### CARBON DIOXIDE[20]—OFFICIAL

**77**

### APPARATUS

Use a 200 cc Erlenmeyer flask closed with a 2-holed stopper; one hole is fitted with a dropping funnel, the stem of which extends almost to the bottom of the flask, and the outlet of a condenser, which is inclined upward at an angle of 30° from the horizontal, passes downward thru the other hole. The upper end of the condenser is connected with a $CaCl_2$ tube, which in turn is connected with a double U-tube filled in the middle with pumice fragments, previously saturated with $CuSO_4$ soln (20% $CuSO_4.5H_2O$) and subsequently dehydrated, and with $CaCl_2$ at either end. Then follow two weighed U-tubes for absorbing the $CO_2$, the first filled with porous soda-lime, and the second, $\frac{1}{3}$ with soda-lime and $\frac{2}{3}$ with $CaCl_2$, the latter reagent being placed at the exit end of the train. A Geissler bulb, partly filled with $H_2SO_4$ is attached to the last U-tube to show the rate of gas flow. An aspirator is connected with the Geissler bulb to draw air thru the apparatus. An absorption tower filled with soda-lime is connected with the mouth of the dropping funnel to remove $CO_2$ from the air entering the apparatus.

### 78 DETERMINATION

Weigh 2 g of the powder or 10 g of the paste into the Erlenmeyer flask; add about 20 cc of $H_2O$; attach the flask to the apparatus, omitting the 2 weighed U-tubes; and draw $CO_2$-free air thru the apparatus until the original air is displaced. Then attach the weighed U-tubes in position, as described under **77**; close the stopcock of the dropping funnel; pour into it 50 cc of HCl (1+4); reconnect with the soda-lime tower; and allow the acid to flow into the Erlenmeyer flask, slowly if there is much $CO_2$, rapidly if there is little. When effervescence diminishes, place a low Bunsen flame under the flask and start a flow of $H_2O$ thru the condenser, allowing a slow current of air to flow thru the apparatus at the same time. Maintain a steady but quiet ebullition and a slow air current thru the apparatus. Boil for a few min. after the $H_2O$ has begun to condense in the condenser, then remove the flame and continue the aspiration of air at the rate of about 2 bubbles per second until the apparatus is cool. Disconnect the weighed absorption tubes, cool in the balance case, and weigh. The increase in weight is $CO_2$.

### COPPER

### 79 *Electrolytic Method—Official*

Dissolve 2 g of the powdered sample in 25 cc of $HNO_3$ (1+4), dilute to 100 cc, and electrolyze, using a rotating spiral anode and a current of about 3 amperes, as directed under **16**, beginning with "Wash into a weighed 150 cc Pt dish."

### 80 *Thiosulfate Method—Official*

Dissolve 2 g of the powdered sample in about 25 cc of $HNO_3$ (1+4), dilute to 50 cc, add $NH_4OH$ in excess, and heat; then, without removing the precipitate which is formed, boil off the excess of $NH_3$, add 3–4 cc of acetic acid, cool, add 10 cc of 30% KI soln, and titrate as directed under **17**, beginning with "titrate with standard thiosulfate soln."

## BORDEAUX MIXTURE WITH PARIS GREEN

### 81 MOISTURE—OFFICIAL

Proceed as directed under **76**.

### 82 CARBON DIOXIDE—OFFICIAL

Proceed as directed under **78**.

### 83 TOTAL ARSENIC—OFFICIAL

Proceed as directed under **5** or **8**, using 2 g of the sample and calculating the results as $As_2O_3$.

### 84 TOTAL ARSENIOUS OXIDE—OFFICIAL

Proceed as directed under **23**, using 0.5–1.0 g of the sample.

### 85 WATER-SOLUBLE ARSENIOUS OXIDE—OFFICIAL

Proceed as directed under **29**, using 2 g of the sample and slightly acidifying the aliquot employed with HCl (1+4) before adding the excess of $NaHCO_3$.

### COPPER

**86** *Electrolytic Method I—Official*

Proceed as directed under **16.**

**87** *Electrolytic Method II—Official*

Dissolve 2 g of the powdered sample in a 150 cc beaker with 5 cc of $HNO_3$, add 25 cc of a 3% soln of $H_2O_2$, and warm on a steam bath for 5–10 min. Add 25 cc more of $H_2O_2$ soln; dilute to 100 cc; and electrolyze, using a weighed gauze cathode, a rotating paddle anode, and a current of 2-3 amperes. At the end of about 20 min., add 15–20 cc more of the $H_2O_2$ soln. After all Cu is deposited, which should not require more than 45 min., and while the current is still flowing, wash the deposit with $H_2O$ by siphoning. Then interrupt the current, rinse with alcohol, dry for a few min. in an oven, weigh, and calculate the percentage of Cu. (Do not pass the current for more than 5-10 min. after all Cu is deposited without adding more of the $H_2O_2$ soln.)

**88** *Thiosulfate Method—Official*

Proceed as directed under **17.**

## BORDEAUX MIXTURE WITH LEAD ARSENATE

**89** MOISTURE—OFFICIAL

Proceed as directed under **76.**

**90** CARBON DIOXIDE—OFFICIAL

Proceed as directed under **78.**

**91** TOTAL ARSENIC—OFFICIAL

Proceed as directed under **5** or **8,** using 2 g of the sample and calculating the results as $As_2O_5$.

**92** WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under **13** and calculate the results as $As_2O_5$.

### COPPER

**93** *Electrolytic Method—Official*

Proceed as directed under **16.**

**94** *Thiosulfate Method—Official*

Proceed as directed under **17.**

**95** LEAD OXIDE—OFFICIAL

Proceed as directed under **15.**

## BORDEAUX MIXTURE WITH CALCIUM ARSENATE

**96** MOISTURE—OFFICIAL

Proceed as directed under **76.**

**97** CARBON DIOXIDE—OFFICIAL

Proceed as directed under **78.**

### 98 TOTAL ARSENIC—OFFICIAL

Proceed as directed under **5** or **8**, using 2 g of the sample and calculating the results as $As_2O_5$.

### 99 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under **13** and calculate the results as $As_2O_5$.

## COPPER

### 100 *Electrolytic Method I—Official*

Proceed as directed under **16.**

### 101 *Electrolytic Method II—Official*

Proceed as directed under **87.**

### 102 *Thiosulfate Method—Official*

Proceed as directed under **17.**

## SODIUM AND POTASSIUM CYANIDES

### CYANOGEN[21]—OFFICIAL

### 103 REAGENTS

**(a)** *Silver nitrate soln.*—0.1 *N*. Standardize against pure NaCl by titration, using chromate indicator; or gravimetrically, weighing the chloride.

**(b)** *Lead carbonate.*

**(c)** *Sodium hydroxide soln.*—Dissolve 100 g of NaOH in $H_2O$ and dilute to 1 liter.

**(d)** *Potassium iodide.*—Crystals, or a saturated soln.

### 104 DETERMINATION

Break the sample into small lumps in a mortar (do not grind). Weigh quickly about 5 g in a weighing bottle and wash into a 500 cc volumetric flask containing about 200 cc of $H_2O$. Add a little $PbCO_3$ to precipitate any sulfides that may be present, dilute to the mark with $H_2O$, mix thoroly, and filter thru a dry filter. Transfer a 50 cc aliquot to a 400 cc beaker; add 200 cc of $H_2O$, 5 cc of the NaOH soln, and 10 drops of the KI soln (or a few crystals); and titrate to a faint opalescence with the 0.1 *N* $AgNO_3$ soln. (In making this titration, it is advantageous to have the beaker over a black surface.) From the number of cc of 0.1 *N* $AgNO_3$ soln used calculate the percentage of CN in the sample. The reaction is represented by the equation: $2NaCN + AgNO_3 = NaCN.AgCN + NaNO_3$; hence, 1 cc of 0.1 *N* $AgNO_3$ soln = 0.005202 g of CN.

## CHLORINE[22]

### *Method I—Official*

### 105 REAGENTS

**(a)** *Silver nitrate soln.*—0.1 *N*. Prepare as directed under **103(a)**.

**(b)** *Ammonium or potassium thiocyanate soln.*—0.1 *N*. Adjust by titrating against the 0.1 *N* $AgNO_3$ soln.

**(c)** *Formaldehyde soln.*—40% chloride-free.

**(d)** *Ferric indicator.*—A saturated soln of ferric ammonium alum.

### 106 DETERMINATION

Transfer a 50 cc aliquot of the soln prepared as directed under **104** to a beaker, dilute with an equal volume of $H_2O$, add 1–2 cc of the HCHO soln, stir well, and let stand for 15 min. Acidify with $HNO_3$ (1+1) (5 cc is usually enough), add a measured volume of the 0.1 *N* $AgNO_3$ soln sufficient to give an excess, stir well, filter, wash, and titrate the excess of Ag in the combined filtrate and washings with the 0.1 *N* thiocyanate soln, using ferric indicator. From the number of cc of 0.1 *N* $AgNO_3$ soln, less the number of cc of 0.1 *N* thiocyanate soln used, calculate the percentage of Cl in the sample.

### *Method II*[22]*—Official*

### 107 REAGENTS

The reagents and solns used are described under **105.**

### 108 DETERMINATION

Transfer a 50 cc aliquot of the soln prepared as directed under **104** to a distilling flask, dilute to 100–150 cc, acidify with a slight excess of acetic acid, and distil, passing the vapors thru a condenser, the delivery end of which dips into a soln of NaOH, to absorb the HCN. After all the HCN has been driven off, which should be the case when 50 cc of distillate has passed over, wash the liquid remaining in the distilling flask into a beaker, add 5 cc of $HNO_3$ (1+1) and then a measured volume of the 0.1 *N* $AgNO_3$ soln sufficient to give an excess, stir well, filter, wash, and titrate the excess of Ag in the combined filtrate and washings with the 0.1 *N* thiocyanate soln, using ferric indicator. From the number of cc of 0.1 *N* $AgNO_3$ soln, less the number of cc of 0.1 *N* thiocyanate soln used, calculate the percentage of Cl in the sample.

## CALCIUM CYANIDE

### CYANOGEN[23]—OFFICIAL

### 109 REAGENTS

(a) *Silver nitrate soln.*—0.1 *N*. Prepare as directed under **103(a)**.

(b) *Soda-lead.*—Dissolve 20 g of Pb-acetate in $H_2O$, dilute to 1 liter, and add 200 g of chloride-free $Na_2CO_3$.

(c) *Sodium hydroxide soln.*—Prepare as directed under **103(c)**.

(d) *Potassium iodide.*—Crystals, or a saturated soln.

### 110 DETERMINATION

Place about 200 cc of $H_2O$ in a 500 cc volumetric flask and carefully dry the neck of the flask. Weigh about 5 g of the sample in a weighing bottle and transfer to the flask with the least possible exposure to the air. Wash the mixture down into the flask and mix by whirling until soln is complete and the small quantity of $CaC_2$ has been decomposed. Then add 25 cc of the soda-lead reagent, or a quantity sufficient to remove sulfides; close the flask with a rubber stopper; and shake thoroly, preferably for half an hour. Dilute to the mark, mix, and filter thru a dry filter. Transfer a 50 cc aliquot to a 400 cc beaker and proceed as directed under **104**, beginning with "Add 200 cc of $H_2O$." 1 cc of 0.1 *N* $AgNO_3$ soln = 0.005202 g of CN. If the percentage of $Ca(CN)_2$ is desired, multiply the percentage of CN by the factor 1.7703.

### CHLORINE[23]

#### *Method I—Official*

**111** REAGENTS

The reagents and solns used are described under **105**.

**112** DETERMINATION

Transfer a 50 cc aliquot of the soln prepared as directed under **110** to a beaker, and proceed as directed under **106**.

#### *Method II[23]—Official*

**113** REAGENTS

The reagents and solns used are described under **105**.

**114** DETERMINATION

Transfer a 50 cc aliquot of the soln prepared as directed under **110** to a distilling flask, and proceed as directed under **108**.

## SOAP

### MOISTURE[24]—OFFICIAL

**115** *Xylene Distillation Method*

REAGENTS

(a) *Xylene.*—The technical grade is satisfactory for this determination.

(b) *Rosin.*—This should be in the form of lumps, as powdered rosin contains an appreciable quantity of moisture.

**116** DETERMINATION

Weigh about 20 g of the sample into a 300–500 cc flask; add 50 cc of xylene; and, to prevent foaming, add about 10 g of lump rosin. Distil into a Dean and Stark type distilling tube receiver[25] and continue the distillation until no more $H_2O$ collects in the receiver. Allow the contents of the tube to cool to room temp., read the volume of $H_2O$ under the xylene in the tube, and from this volume calculate the percentage of $H_2O$ in the sample.

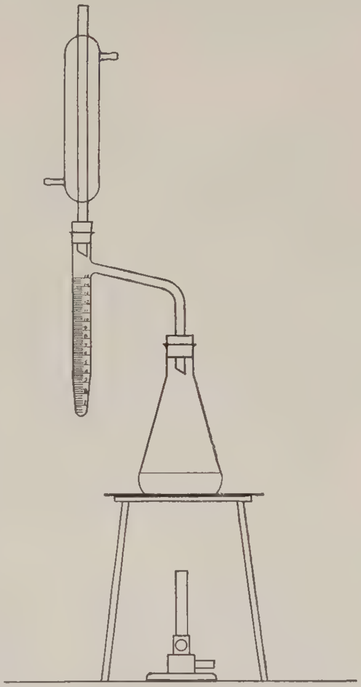


FIG. 5.—DEAN AND STARK DISTILLING TUBE RECEIVER.

### **117** POTASSIUM AND SODIUM[26]—OFFICIAL

Dissolve about 5 g of the soap in $H_2O$, decompose with HCl (1+4), filter off the $H_2O$, and wash the fat with cold $H_2O$. Determine both K and Na in the filtrate as directed under **XII, 16.**

## MINERAL OILS

### UNSULFONATED RESIDUE[27]—OFFICIAL

**118** REAGENTS

*Sulfuric acid.*—38 *N*. Prepare as directed under **VIII, 9.**

119

### DETERMINATION

With a pipet measure 5 cc of the oil into a Babcock cream bottle about 15 cm (6 inches) long, either the 9 g 50% or the 18 g 30% type. With heavy oils, to reduce the viscosity, warm the pipet after a preliminary draining by drawing it several times thru the flame of a Bunsen burner and then drain thoroly. If greater accuracy is desired, weigh the measured charge and calculate its exact volume from the weight and sp. gr. of the oil. Add slowly 20 cc of the $38\ N\ H_2SO_4$, gently shaking or rotating the bottle and taking care that the temp. does not rise above 60°. Cool in ice $H_2O$ if necessary. When the mixture no longer develops heat on shaking, agitate thoroly, place the bottle in a water bath, and heat at 60–65° for 10 min, keeping the contents of the bottle thoroly mixed by shaking vigorously for a period of 20 seconds at 2 min. intervals. Remove the bottle from the bath and fill with $H_2SO_4$ until the oil rises into the graduated neck. Centrifugalize for 5 min. (or longer if necessary to obtain a constant volume of oil) at 1200–1500 r.p.m. Read the volume of unsulfonated residue from the graduations on the neck of the bottle and, to convert to cc, multiply the reading from the 9 g 50% bottle by 0.1 and that from the 18 g 30% bottle by 0.2. From the result thus obtained calculate the percentage by volume of the unsulfonated oil.

## MINERAL OIL—SOAP EMULSIONS

120

### WATER[28]

*Xylene Distillation Method—Official*

Weigh about 25 g of the sample into a 300–500 cc flask; add 50 cc of xylene and, if necessary to prevent foaming, a small piece of rosin; and proceed as directed in **116**, beginning with "Distil into a Dean and Stark distilling tube receiver."

121

### TOTAL OIL[29]—OFFICIAL

Weigh about 10 g of the sample into a Babcock cream bottle. Dilute with about 10 cc of hot $H_2O$ and add 5–10 cc of $H_2SO_4(1+1)$. Set the bottle in a hot water bath for about 5 min. to hasten the separation of the oil, add sufficient saturated NaCl soln to bring the oil layer within the graduations on the neck of the bottle, whirl at a rate of 1200 r.p.m. for 5 min., and allow to cool. Read the volume of the oil layer, determine its density, and from these values calculate its weight and percentage From this percentage value, deduct the percentage of fatty acids (and phenols if present) determined separately, to obtain the percentage of oil in the sample.

122

### SOAP[28]—OFFICIAL

(In this method error will result if the apparent molar weight of the fatty acids varies appreciably from that of oleic acid.)

Weigh 20 g of the sample into a separatory funnel, add 60 cc of petroleum ether, and extract the mixture once with 20 cc and 4 times with 10 cc of 50% alcohol. Break the emulsion if necessary with 1 or 2 cc of a strong soln of NaOH, allowing the soln to run down the side of the separatory funnel, which is then gently twirled and allowed to stand for a few min. Draw off the alcoholic layers and wash them successively thru petroleum ether contained in 2 other separatory funnels. Combine the alcoholic extracts in a beaker and evaporate on a steam bath to remove the alcohol. Dissolve the residue in about 100 cc of $H_2O$ made alkaline with NaOH. Transfer to a separatory funnel, acidify with HCl or $H_2SO_4$, extract 3 times with ether, and wash the ether extracts twice with $H_2O$. Combine the ether extracts, evaporate in a weighed beaker on a steam bath, and weigh as fatty acids. From the weight of fatty acids calculate the percentage of soap in the sample as Na- or K-oleate.

### 123 UNSULFONATED RESIDUE—OFFICIAL

Determine on 5 cc of the recovered oil, as described under Mineral Oils, **119**.

### 124 ASH[30]—OFFICIAL

Evaporate 10 g of the sample, or more if necessary, in a Pt dish; ignite; and leach the charred mass with $H_2O$. Ignite the residue, add the leachings, evaporate to dryness, ignite, and weigh. From this weight calculate the percentage of ash. Test the ash for Cu, Ca, $CaF_2$, etc.

## SODA LYE

### CARBONATE AND HYDROXIDE[31]—OFFICIAL

### 125 REAGENTS

(a) *Hydrochloric acid.*—0.5 *N*. Prepare and standardize as directed under **II, 17**(a).

(b) *Methyl orange indicator.*—Prepare as directed under **3**(f).

(c) *Phenolphthalein indicator.*—Dissolve 1 g of phenolphthalein in 100 cc of neutralized alcohol 95% by volume.

(d) *Barium chloride soln.*—Dissolve 100 g of $BaCl_2.2H_2O$ and dilute to 1 liter.

### 126 DETERMINATION

Weigh about 10 g of the sample from a weighing bottle, dissolve in $CO_2$-free $H_2O$, and dilute to a definite volume. Titrate an aliquot of this soln with the 0.5 *N* HCl, using the methyl orange indicator, and note the total alkalinity thus found. Transfer an equal aliquot to a volumetric flask and add enough of the $BaCl_2$ soln to precipitate all the carbonate, avoiding any unnecessary excess. Dilute to the mark with $CO_2$-free $H_2O$, stopper, shake, and set aside. When the liquid becomes clear, pipet off $\frac{1}{2}$ and titrate with the 0.5 *N* HCl, using the phenolphthalein indicator. The number of cc of 0.5 *N* acid required for this titration, multiplied by 2, gives the number of cc of 0.5 *N* acid equivalent to the NaOH present in the original aliquot. The difference between this figure and the number of cc of 0.5 *N* HCl required for the total alkalinity represents the number of cc of 0.5 *N* acid equivalent to the $Na_2CO_3$ present in the aliquot. Calculate the percentages of $Na_2CO_3$ and NaOH present in the sample.

## TOBACCO AND TOBACCO EXTRACT

### NICOTINE

### *Silicotungstic Acid Method[32]—Official*

### 127 REAGENTS

(a) *Silicotungstic acid soln.*—Dissolve 120 g of silicotungstic acid ($4H_2O.SiO_2.12WO_3.22H_2O$) in $H_2O$ and dilute to 1 liter. (There are several silicotungstic acids. The acids $4H_2O.SiO_2.10WO_3.3H_2O$ and $4H_2O.SiO_2.12WO_3.20H_2O$ do not give crystalline precipitates with nicotine and should not be used.)

(b) *Sodium hydroxide soln.*—Dissolve 400 g of NaOH in $H_2O$ and dilute to 1 liter.

(c) *Hydrochloric acid.*—(1+4).

### 128 DETERMINATION

Weigh such a quantity of the preparations as will contain preferably between 0.1 and 1.0 g of nicotine (if the sample contains very little nicotine, i.e. about 0.1%, do not increase the quantity to the point where it interferes with the distillation); wash with $H_2O$ into a 500 cc round-bottomed distillation flask; and add a little paraffin to prevent frothing, a few small pieces of pumice, and a slight excess of the NaOH soln, using the phenolphthalein indicator. Distil rapidly in a current of steam thru a well-cooled condenser, connected by means of an adapter with a suitable flask containing 10 cc of the dilute HCl. When distillation is well under way, heat the distillation flask to reduce the volume of the liquid as far as practicable without bumping or undue separation of insoluble matter. Distil until a few cc of the distillate shows no cloud or opalescence when treated with a drop of the silicotungstic acid and a drop of the dilute HCl. Confirm the alkalinity of the residue in the distillation flask with the phenolphthalein indicator. Make the distillate, which may amount to 1000–1500 cc, to a convenient volume (the soln may be concentrated on the steam bath without loss of nicotine); mix well; and pass thru a large dry filter if not clear. Test a portion with methyl orange to confirm its acidity. Pipet an aliquot, containing about 0.1 g of nicotine, into a beaker (if the samples contain very small quantities of nicotine, an aliquot containing as little as 0.01 g of nicotine may be used); add to each 100 cc of liquid 3 cc of the dilute HCl, or more if the necessity is indicated by the test with methyl orange, and 1 cc of the silicotungstic acid for each 0.01 g of nicotine supposed to be present. Stir thoroly and let stand overnight. Before filtering, stir the precipitate to see that it settles quickly and is in crystalline form, filter on an ashless filter, and wash with cold HCl (1+1000). Transfer the paper and precipitate to a weighed Pt crucible, dry carefully, and ignite until all C is destroyed. Finally heat over a Teclu or Meker burner for not more than 10 min. The weight of the residue, multiplied by 0.1140, gives the weight of nicotine present in the aliquot.

## FORMALDEHYDE SOLUTIONS

### FORMALDEHYDE

### *Hydrogen Peroxide Method*[33]—*Official*

### 129 REAGENTS

(a) *Sulfuric acid.*—1 *N*. Prepare as directed under **II, 17(b)**.

(b) *Sodium hydroxide.*—1 *N*. Standardize against (a), using the litmus or brom-thymol blue indicator. 1 cc = 30.02 mg. of HCHO.

(c) *Hydrogen peroxide.*—The commercial reagent containing about 3% of $H_2O_2$. If acid, neutralize with the 1 *N* NaOH (b), using litmus or brom-thymol blue indicator.

(d) *Litmus indicator.*—A soln of purified litmus of sufficient strength that 3 drops will impart a distinct blue color to 50 cc of $H_2O$.

(e) *Brom-thymol blue indicator.*—Dissolve 1 g of brom-thymol blue in 500 cc of alcohol, 50% by volume.

### 130 DETERMINATION

Measure 50 cc of the 1 *N* NaOH into a 500 cc Erlenmeyer flask and add 50 cc of the $H_2O_2$. Then add a weighed quantity, about 3 g, of the HCHO soln under examination, allowing the point of the weighing pipet to reach nearly to the liquid in the flask. Place a funnel in the neck of the flask and heat on a steam bath for 5 min., shaking occasionally. Remove from the steam bath, wash the funnel with $H_2O$, cool the flask to about room temp., and titrate the excess NaOH with 1 *N* acid, using the brom-thymol blue or litmus indicator. (It is necessary to cool the flask before titration with the acid in order to obtain a sharp end point with the litmus.) From the amount of 1 *N* NaOH consumed and the weight of the sample calculate the percentage of HCHO, according to the following equation:

$$NaOH+HCHO+H_2O_2=NaCOOH+2H_2O.$$

### *Cyanide Method*[34]*—Official*

(Applicable only to dilute solutions.)

**131** REAGENTS

(a) *Silver nitrate.*—0.1 *N*. Prepare as directed under **XII, 34**(a).
(b) *Ammonium thiocyanate.*—0.1 *N*. Prepare as directed under **XII, 34**(b).
(c) *Potassium cyanide soln.*—Dissolve 3.1 g of KCN in 500 cc of $H_2O$.

**132** DETERMINATION

Treat 15 cc of the 0.1 *N* $AgNO_3$ soln with 6 drops of $HNO_3$ (1+1) in a 50 cc volumetric flask; add 10 cc of the KCN soln; dilute to the mark; shake well; filter thru a dry filter; and titrate 25 cc of the filtrate with the 0.1 *N* $NH_4SCN$, as directed under **XII, 35.** Acidify another 15 cc portion of the 0.1 *N* $AgNO_3$ with 6 drops of the dilute $HNO_3$ and treat with 10 cc of the KCN soln to which has been added a measured quantity (the weight must be calculated from the sp. gr.) of the HCHO soln containing not over 25 mg of HCHO. Dilute to 50 cc, filter, and titrate a 25 cc aliquot with the 0.1 *N* $NH_4SCN$ for the excess of Ag as before. The difference between the number of cc of $NH_4SCN$ used in these 2 titrations, multiplied by 2, gives the number of cc of 0.1 *N* $NH_4SCN$ corresponding to the KCN used by the HCHO. Calculate the percentage of HCHO present. 1 cc of 0.1 *N* $NH_4SCN$ = exactly 3 mg of HCHO.

## LIME-SULFUR SOLUTIONS

### TOTAL SULFUR[35]—OFFICIAL

**133** PREPARATION OF SAMPLE

Weigh about 10 g of the soln, transfer to a 250 cc volumetric flask, and immediately dilute to the mark with recently boiled and cooled $H_2O$. Mix thoroly and transfer to a number of small bottles, filling them completely and avoiding contact of the soln with air as much as possible. Stopper the bottles; seal with paraffin; and preserve in a dark, cool place.

**134** DETERMINATION

(Sulfur-free reagents should be used for all work.)

Dissolve 2–3 g of $Na_2O_2$ in 50 cc of cold $H_2O$ in a 250 cc beaker. Transfer a 10 cc aliquot of the soln, prepared for analysis as directed under **133**, to this aqueous soln of $Na_2O_2$, keeping the tip of the pipet constantly just under the surface of the liquid until necessary to raise it for drainage at the end. Use a clean dry pipet for measuring each portion. Cover the beaker with a watch-glass and heat on a steam bath, with occasional stirring, until all the S is oxidized to sulfate (indicated by the disappearance of the yellow color). Wash off the watch-glass and the sides of the beaker, acidify with HCl (1+4), evaporate to complete dryness, treat with $H_2O$ acidified with HCl, boil, and filter to remove $SiO_2$. Dilute the filtrate to 300 cc, add 50 cc of HCl, heat to boiling, and add 10% $BaCl_2$ soln slowly and with constant stirring. (The $BaCl_2$ should be added at such a rate that about 4 min. is required for running in the necessary quantity, 11 cc for 1 g of $BaSO_4$. The rate is best regulated by attaching a suitable capillary tip to a buret containing the $BaCl_2$ soln.) Evaporate to dryness on a steam bath, take up with hot $H_2O$, filter thru a quantitative filter, wash until free from chlorides, ignite carefully, and heat to constant weight over a

Bunsen burner. Calculate the percentage of S from the weight of $BaSO_4$, using the factor 0.1374.

### MONOSULFIDE EQUIVALENT[6]—TENTATIVE

**135** REAGENT

*Iodine soln.*—0.1 *N*. Prepare as directed under **3(b)**, using 12.7 g of I and 25 g of KI.

**136** DETERMINATION

Dilute 10 cc of the soln, prepared as directed under **133**, to about 30 cc with recently boiled and cooled $H_2O$ and titrate with the 0.1 *N* I soln until the yellow color just disappears. (There should be no difficulty in determining this end point; if there is, a small crystal of Na-nitroprusside may be used. It must not be added until the end point is practically reached, since the blue color, if well developed, cannot be destroyed except by an excess of I.) From the number of cc of 0.1 *N* I used calculate the percentage of monosulfide equivalent. 1 cc of 0.1 *N* I = 0.0016 g of S as monosulfide equivalent.

### THIOSULFATE SULFUR

#### *Zinc Chloride Method*[35]—*Official*

**137** REAGENT

*Ammoniacal zinc chloride soln.*—Dissolve 50 g of pure $ZnCl_2$ in about 500 cc of $H_2O$, add 125 cc of $NH_4OH$ and 50 g of $NH_4Cl$, and dilute to 1 liter.

**138** DETERMINATION

To 50 cc of $H_2O$ in a 200 cc volumetric flask, add, in the manner indicated under **134**, 50 cc of the soln prepared as directed under **133**. Add a slight excess of the ammoniacal $ZnCl_2$ soln and dilute to the mark. Shake thoroly and filter thru a dry filter. To 100 cc of the filtrate add a few drops of methyl orange or methyl red indicator and exactly neutralize with 0.1 *N* HCl. Titrate the neutral soln with the approximately 0.05 *N* I soln, **3(b)**, using a few drops of the starch indicator, **3(e)**. From the number of cc of I soln used calculate the percentage of thiosulfate S present. As the value of the I soln is given in terms of $As_2O_3$ it is necessary to multiply this value by 1.296 to obtain the equivalent of thiosulfate S.

#### **139** *Iodine Titration Method*[36]—*Tentative*

Continue the titration of the soln used in the determination of the monosulfide equivalent, **136**, with the 0.1 *N* I soln, letting the I act as its own indicator until a small drop produces a slight permanent coloration. From the number of cc of 0.1 *N* I used calculate the percentage of thiosulfate S. 1 cc of 0.1 *N* I = 0.0064 g of S as thiosulfate.

### SULFIDE SULFUR

#### **140** *Zinc Chloride Method*[35]—*Official*

To 10–15 cc of $H_2O$ in a small beaker add, in the manner indicated under **134**, a 10 cc aliquot of the soln prepared as directed under **133**. Calculate the quantity of ammoniacal $ZnCl_2$ soln, **137**, necessary to precipitate all the S in the aliquot and add a slight excess. Stir thoroly, filter, wash the precipitate twice with cold $H_2O$, and transfer the filter paper and precipitate to the beaker in which the precipitation was made. Cover with $H_2O$; disintegrate the paper with a glass rod; and add about

3 g of $Na_2O_2$, keeping the beaker well covered with a watch-glass. Warm on a steam bath with frequent shaking until all the S is oxidized to sulfate, adding more $Na_2O_2$ if necessary. Make slightly acid with HCl (1+4), filter to remove shreds of the filter paper, wash thoroly with hot $H_2O$, and determine the S in the filtrate as directed under **134.**

### 141 *Iodine Titration Method*[36]*—Tentative*

Allow the soln from **139** to stand several hours with occasional stirring, or acidify with a few drops of HCl (1+4); warm gently with stirring; filter; and wash thoroly with warm $H_2O$. Place the filter paper with the S in a small vessel and dissolve the S in about 15 cc of NaOH soln, **3**(d), by heating gently on a steam or water bath for 1–1.5 hours (do not boil). Keep the flask covered and shake gently a few times during the digestion to remove the S from the sides. Oxidize by adding 2–3 g of $Na_2O_2$ dissolved in 50 cc of cold $H_2O$ and complete the determination as directed under **134,** beginning with "Cover the beaker with a watch-glass."

### 142 *Indirect Method—Tentative*

The difference between the total S and the sum of the thiosulfate S and sulfate S is the sulfide S.

## SULFATE SULFUR

### 143 *Zinc Chloride Method—Official*

Slightly acidify the soln from the determination of thiosulfate S, **138,** with HCl (1+4), heat to boiling, add slowly and with constant stirring a slight excess of a 10% $BaCl_2$ soln, boil for 30 min., allow to stand overnight, filter, calculate the S from the weight of $BaSO_4$, and report as % of sulfate S.

### 144 *Iodine Titration Method*[36]*—Tentative*

To the filtrate from the determination of thiosulfate S, **139,** add several drops of HCl, precipitate in the cold with 5 cc of a 10% $BaCl_2$ soln, allow to stand overnight, filter, calculate the S from the weight of $BaSO_4$, and report as percentage of sulfate S.

### 145 TOTAL LIME[35]—OFFICIAL

To 25 cc of the soln, prepared as directed under **133,** add 10 cc of HCl, evaporate to dryness on a steam bath, treat with $H_2O$ and a few cc of HCl (1+4), warm until all the $CaCl_2$ is dissolved, and filter to remove S and any $SiO_2$ that may be present. Dilute the filtrate to a volume of 200–250 cc, heat to boiling, add a few cc of $NH_4OH$ in excess, and then an excess of a saturated soln of $(NH_4)_2C_2O_4$. Continue the boiling until the precipitated $CaC_2O_4$ assumes a well defined granular form, allow to stand for an hour, filter, and wash a few times with hot $H_2O$. Ignite in a Pt crucible over a blast lamp to constant weight and calculate to percentage of CaO.

## FLUORINE

### *By Volatilization*[37]*—Tentative*

### 146 REAGENTS

(a) *Sulfuric acid.*—98–98.5%. Prepare by one of the following methods:

(1) Add sufficient fuming $H_2SO_4$ to ordinary concentrated $H_2SO_4$ to give a soln containing about 99%, as determined by titration. Heat in an open casserole for 1 hour after the appearance of dense fumes. Adjust the acid to contain 98–98.5% by

the addition of either ordinary or fuming $H_2SO_4$, as determined by titration. Protect the acid from atmospheric moisture.

(2) Boil ordinary (approximately 95%) $H_2SO_4$ in an open casserole to about $\frac{2}{3}$ its original volume. Determine the strength by titration. Protect the acid from atmospheric moisture.

(3) Heat ordinary (approximately 95%) $H_2SO_4$ in a retort until about 1/10 of the original volume has boiled off. Determine its strength and protect from the atmosphere as usual.

**(b)** *Quartz flour* (silica).—Grind ordinary quartz to 200-mesh and ignite at a red heat.

**(c)** *Silver sulfate in sulfuric acid.*—Dissolve 10 g of pure, dry $Ag_2SO_4$ in 100 cc of $H_2SO_4$ prepared as in **(a)**.

**(d)** *Chromium trioxide in sulfuric acid.*—Add finely powdered $CrO_3$, previously dried at 110°, to 100 cc of $H_2SO_4$ prepared as in **(a)**, permitting an excess of the powder to remain in suspension.

**(e)** *Asbestos.*—Ignite 5 g of acid-washed asbestos of medium fiber to a red heat.

**(f)** *Sodium hydroxide soln.*—0.1 *N.*

NOTE.—HCl, oxides of N, and S are absorbed, the former in $Ag_2SO_4$ and the latter in $CrO_3$-$H_2SO_4$. Asbestos contained in tube *I* serves to condense traces of $SO_3$ that may escape absorption in the $CrO_3$-$H_2SO_4$ soln.

## 147 APPARATUS

(*A*), wash bottle containing $H_2SO_4$ for initial drying of the ingoing air. (*B*), jar filled with 4-mesh, granular $CaCl_2$. (*C*), jars loosely filled with $P_2O_5$, with tops and bottoms protected by layers of ignited asbestos. (*D*), reaction flask detailed in Fig. 7. (*E*), metal air bath, with asbestos board cover to protect the upper part of the flask. (*F*), gas burner (a hot plate or furnace may be substituted). (*G*), Schmitz tube (4-inch) containing glass beads in one arm and 5 cc of $Ag_2SO_4$ soln in $H_2SO_4$ in the other arm. (*H*), Bowen bulbs containing 25 cc of $CrO_3$ in $H_2SO_4$. (*I*), glass tube (10 x $\frac{3}{8}$ inches) containing ignited asbestos, as illustrated. (*J*), Meyer absorption bulb containing 50 cc of $H_2O$ for collection of the $SiF_4$. (*K*), pressure regulator containing Hg. (It is imperative that joints be made air-tight by bringing all apparatus tubes in close contact within rubber tubing connectors.)

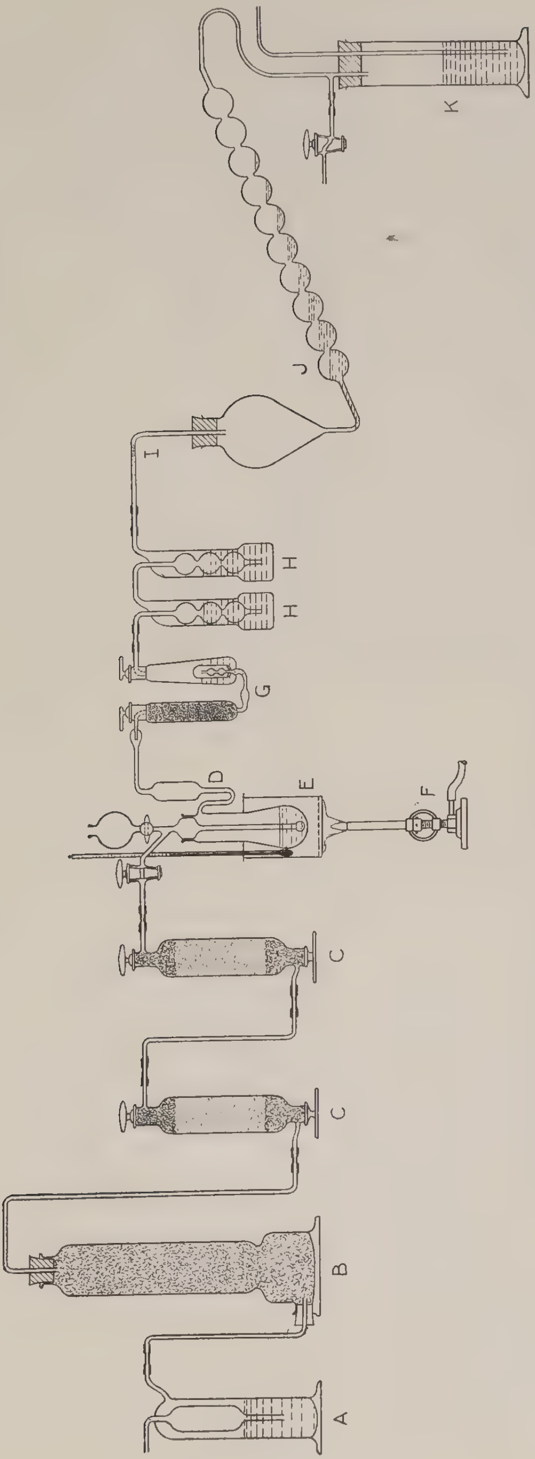
## 148 PRELIMINARY TREATMENT OF SAMPLE

(1) If organic matter is present, weigh into a Pt dish a quantity of the finely ground material equivalent to about 30-40 mg of F. Add about 0.5 g of pulverized CaO and sufficient $H_2O$ to form a smooth paste, mix thoroly, dry, and ignite in a muffle furnace at 500° until a white ash is obtained. Protect in a desiccator.

(2) For substances that contain no organic matter proceed without preliminary treatment other than pulverizing and drying. (The method should not be used for the determination of F in substances containing B.)

## 149 DETERMINATION

Place the sample as prepared under (1) or (2), together with 1 g of 200-mesh quartz flour, in an agate mortar, and mix thoroly. Transfer the mixture thru a powder funnel to the dry reaction flask (*D*), (Fig. 6) and place the flask in its position in the train. Add 50 cc of $H_2O$ to the Meyer absorption bulb (*J*), and connect as illustrated. Apply suction and permit a current of air to pass thru the apparatus at the rate of 3 bubbles per second, for a period of 30 min. Maintain this rate of aeration throughout the entire determination. Add thru the funnel of the reaction


A
B
C
C
D
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F
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H
I
J
K

FIG. 6.—APPARATUS FOR THE DETERMINATION OF FLUORINE BY THE VOLATILIZATION METHOD.

flask about 10 cc of anhydrous $H_2SO_4$ (reagent a), retaining in the funnel a few drops of the acid. If the ash content of the sample is high, add the acid very slowly. Allow to stand 10 min. and shake at least twice during this interval. Apply heat to the reaction flask, slowly bringing the temp. to about 220° (as indicated by a thermometer supported in a position such that its Hg-bulb is in contact with the flask at a point below the surface of the acid), and maintain this temp. for 1 hour. Shake the flask every 5 min. until the gray-white scum, which forms on the surface, completely disappears. After 1 hour increase the temp. and boil for a period of 5 min. Remove the heat but continue to aerate for 30 min. to recover any $SiF_4$ remaining in the system. Disconnect the absorption bulb (a short auxiliary tube of glass with stopcock connecting (*I*) and (*J*) is advantageous in preventing back pressure while (*J*) is being disconnected) and transfer its contents with rinsings to a 500 cc Erlenmeyer flask. Dilute with $H_2O$ to about 250 cc, heat to boiling, and boil gently for 5 min. Titrate the hot soln with 0.1 *N* NaOH, using phenolphthalein as indicator. 1 cc of 0.1 *N* NaOH = 0.0019 g of F. Conduct a blank determination and correct for the volume of 0.1 *N* alkali required in the blank.

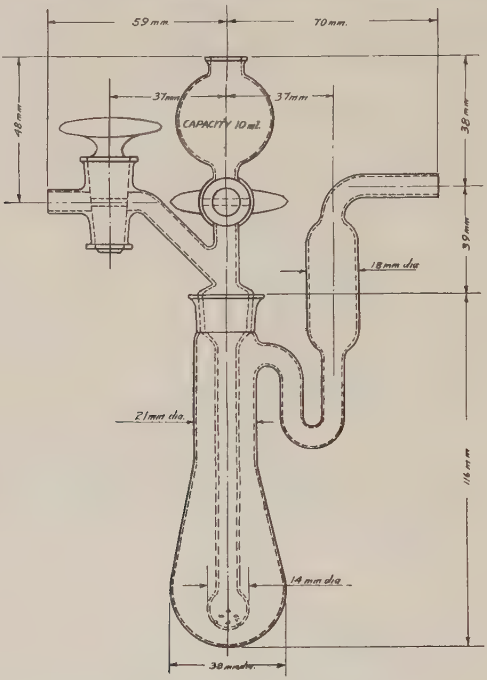

FIG. 7.—REACTION FLASK FOR THE DETERMINATION OF FLUORINE

## ORGANIC MERCURIAL SEED DISINFECTANTS

### MERCURY

*Volatilization Method*[38]*—Official, First Action*

150 REAGENTS

(a) *Anhydrous sodium carbonate.*
(b) *Powdered barium carbonate.*
(c) *Alcohol,* 95 *per cent.*

**151**

### APPARATUS

The apparatus used (Fig. 8) consists of 2 flanged crucibles that can be clamped mouth to mouth by means of 2 rings and holding screws. The lower crucible is made of Fe and sets in an asbestos board with a hole just large enough to receive it; the upper one is of Au, and the opening is slightly larger than that of the other so that there will be no tendency for the Hg to lodge in the joint between the two flanges. The Au crucible is fitted with a cooling device by which $H_2O$ may be slowly circulated thru a large tube attached to it by Gooch tubing.

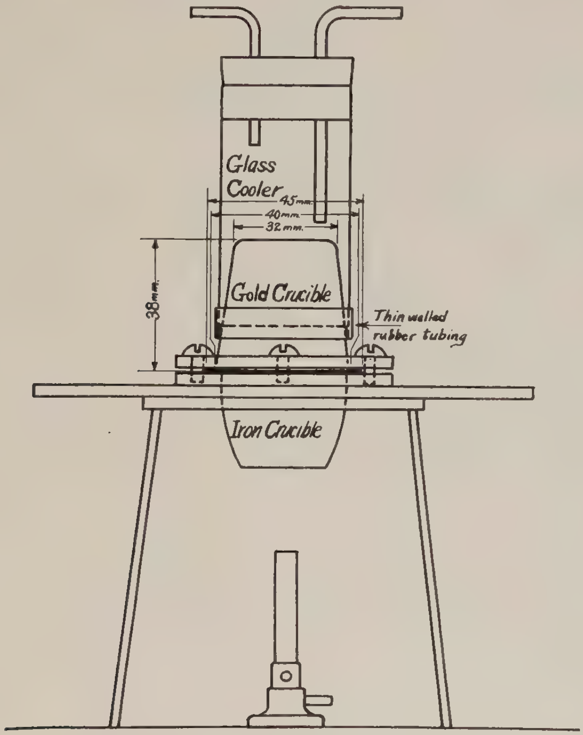

FIG. 8.—APPARATUS TO BE USED IN VOLATILIZATION METHOD

**152**

### DETERMINATION

Weigh 1 g of the sample into the Fe crucible and mix it thoroly with 5 g of anhydrous $Na_2CO_3$. Cover the mixture with a thin layer of $Na_2CO_3$ and then with 10 g of finely powdered $BaCO_3$. Put the weighed Au crucible in place, clamp the two together, set the Fe crucible in place in the asbestos board, start the cooling $H_2O$, and gently heat the Fe crucible. Do not run the $H_2O$ too fast because the Hg amalgamates best with the Au crucible if the temp. is allowed to rise to about 50°. Heat below red heat for 30 min., cool, remove the Au crucible, wash it with 95% alcohol, dry with the heat of the hand, and then place in a $CaCl_2$ desiccator until it attains constant weight. Calculate the increase in weight of the Au crucible as percentage of metallic Hg in the sample. If the product contains more than 12% Hg, less than 1 g should be used, since 0.12 g of Hg is about all that the Au crucible can safely retain. Remove the Hg from the Au crucible, preparatory to another experiment, by a short ignition at a dull red heat under a hood having a good draught. (The crucible will melt in the full heat of a Bunsen burner.)

*Precipitation Method*[38]*—Official, First Action*

**153** REAGENTS

(a) *Hydrogen peroxide.*—A 30% soln, commonly designated as "perhydrol" or "superoxol."

(b) *Potassium permanganate soln.*—Approximately 0.1 *N*.

**154** APPARATUS

*Digestion flask.*—A 200 cc Erlenmeyer flask, fitted with an air condenser by means of a ground-glass joint.

**155** DETERMINATION

Place 0.5–2.0 g of the sample, depending on the quantity of Hg present, in the digestion flask. Add 10 cc of $H_2SO_4$, connect the flask to the condenser, and rotate in order to bring all the sample into contact with the acid. Then add dropwise thru the condenser tube 3–5 cc of the 30% $H_2O_2$ soln, and mix by rotation of the flask. After the active reaction has subsided, heat over a low flame for 15–20 min., add 5 cc more of the $H_2O_2$ and continue the heating until all organic matter is destroyed (indicated by a clear soln), adding more $H_2O_2$ if necessary. Remove the flask from the heat, allow to cool, wash down the condenser, and transfer the contents to a beaker, filtering if necessary. Dilute to about 200 cc and destroy the excess of $H_2O_2$ by titration with $KMnO_4$ soln. Precipitate the Hg with $H_2S$, filter thru a weighed Gooch crucible, and dry the precipitate in the oven at 105–110°. Extract the dried precipitate with $CS_2$ to remove any precipitated S, again dry, and weigh. From the weight of HgS calculate the percentage of metallic Hg, using the factor 0.86219.

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# VII. CAUSTIC POISONS

## PHENOL

### *Method I*[1]*—Official*

(Applicable to the determination of phenol in commercial cresols, saponified cresol solns, coal tar dips and disinfectants, and to kerosene solns of phenols except in the presence of salicylates or betanaphthol.)

### 1 REAGENTS

(a) *Dilute nitric acid.*—Blow air thru $HNO_3$ until it is colorless and dilute 1 volume of this acid with 4 volumes of $H_2O$.

(b) *Millon's reagent.*—Treat 2 cc of Hg in a 200 cc Erlenmeyer flask with 20 cc of $HNO_3$. Place the flask under a hood, and after the first violent reaction is over shake as much as necessary to effect subdivision of the Hg and maintain action. After about 10 min., when the action has practically ceased even in the presence of undissolved Hg, add 35 cc of $H_2O$, and if basic salt separates, add a sufficient quantity of the dilute $HNO_3$ to dissolve it. Add a 10% soln of NaOH dropwise with thoro mixing until the curdy precipitate that forms after the addition of each drop no longer redissolves but disperses to an evidently permanent turbidity. Then add 5 cc of the dilute $HNO_3$ and mix well. As the soln deteriorates, do not use it later than the day of preparation.

(c) *Standard phenol.*—Prepare a stock soln by dissolving a weighed quantity of the pure substance (congealing point not lower than 40°) in a sufficient quantity of $H_2O$ to make not less than a 1% soln. From this stock soln make a 0.025% soln in additional distilled $H_2O$. (This second soln, which constitutes the final standard, should be prepared on the day of use.)

(d) *Dilute formaldehyde soln.*—Dilute 2 cc of commercial 37% HCHO soln to 100 cc with distilled $H_2O$.

### 2 APPARATUS

(a) *Nessler cylinders.*—50 cc tall form, matched.

(b) *Test tubes.*—Approximately 180 mm x 20 mm, provided with rubber stoppers and marked at 25 cc.

(c) *Water bath for heating the test tubes.*—A beaker containing a disk of wire gauze raised somewhat from the bottom may be used.

### 3 PREPARATION OF SAMPLE

*Commercial Cresol.*—Weigh by difference about 2.5 g of sample into a 250 cc volumetric flask, dissolve in 10 cc of a 10% NaOH soln, and make to the mark with $H_2O$.

*Saponified Cresol Solns, Coal Tar Dips and Disinfectants, Kerosene Solns of Phenols, Etc.*—Weigh by difference about 5 g (or use 5 cc and calculate the weight from the density of the sample) of sample into a 250 cc volumetric flask and dilute to the mark with $H_2O$. In products consisting largely of kerosene, bring the $H_2O$ level to the mark and take aliquots from the aqueous portion only.

### 4 DETERMINATION

Transfer a 5 cc aliquot of the prepared soln to a 200 cc volumetric flask shortly before the determination is to be carried out, dilute to about 50 cc, add 1 drop of methyl orange indicator soln and then dilute $HNO_3$ (a) until the soln is practically neutral, make to volume, and shake well.

Place 5 cc of the diluted soln in each of 2 of the marked test tubes, and in each of 2 additional test tubes place 5 cc of the standard phenol soln (**c**). Next flow 5 cc of Millon's reagent (**b**) down the side of each tube, mix, and place the tubes in a bath of boiling water; continue the boiling for exactly 30 min.; cool immediately and thoroly by immersion in a bath of cold $H_2O$ for at least 10 min., and then add 5 cc of dilute $HNO_3$ (**a**) to each tube.

Mix well and add 3 cc of dilute HCHO soln, **1** (**d**), to one of each pair of tubes; make all the tubes to the 25 cc mark with $H_2O$, stopper them, shake well, and put aside to stand overnight. The next day the contents of the tubes to which HCHO was added will have faded to a yellow, while the others will show an orange or red tint.

Pipet 20 cc from each of the 2 phenol tubes and transfer to 100 cc volumetric flasks; treat each with 5 cc of the dilute $HNO_3$ (**a**), make to the mark, and mix. The red flask contains the "phenol standard," and the yellow flask the "phenol blank." Transfer these solns to burets. Pipet 10 cc of each sample soln into Nessler tubes. (The orange or red constitutes the "unknown" and the yellow the "sample blank," and each Nessler tube must be distinctly marked to avoid confusion.) Next add to the "sample blank" tube a measured quantity of "phenol standard" from its buret and add the same volume of "phenol blank" to the "unknown," thoroly agitate (aided by insertion of the rubber stoppers if necessary), and compare the colors. When the tubes have in this way been brought to a match, each cc of the phenol standard employed is equivalent to 1% of phenol if a portion of sample weighing exactly 5 g was used, or 2% if exactly 2.5 g was used.

Note.—In using this method the following precautions should be borne in mind: A pair of phenol tubes affords sufficient final solns for assaying several unknowns, but all the latter must have accompanied the phenol solns thruout the entire process with identical reagents and treatment. If the end point has been inadvertently overrun it is possible to work back to it, but since mistakes are easy to make in this procedure it is better to repeat the comparison on fresh portions from the original tubes. Too much delay in matching the tubes must be avoided after the titration has been started, otherwise the excess of HCHO present in the blanks may have time after mixture to affect the intensity of the red color.

Millon's reagent is dangerously poisonous and should not be transferred with an ordinary pipet and mouth suction unless a protective trap of some kind is used.

### *Method II*[2]*—Official*

(Applicable to the determination of phenol in the presence of salicylates.)

### 5 REAGENTS

The reagents and solns used are described under **1**.

### 6 DETERMINATION

Weigh by difference into a separatory funnel approximately 10 g of sample (or use 10 cc and calculate the weight from the density of the sample). Add 50 cc of kerosene and extract 3 times with 100 cc portions of $H_2O$. Filter the aqueous extracts thru a wet filter into a 500 cc volumetric flask, make to volume with distilled $H_2O$, and proceed as directed under 4, beginning with "Transfer a 5 cc aliquot of this soln to a 200 cc volumetric flask."

When the tubes have been brought to a match, each cc of the phenol standard employed is equivalent to 1% of phenol if a portion of the sample weighing exactly 10 g was used.

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[2] Ind. Eng. Chem., Anal. Ed., **1**, 232 (1929).

# VIII. NAVAL STORES

## ROSIN

### TOLUOL-INSOLUBLE MATERIAL IN ROSIN[1]—TENTATIVE

1 PREPARATION OF SAMPLE

(1) If the sample is less than 200 g, immediately before the determination is made powder it to pass a standard 10-mesh sieve, mix thoroly, and place in a wide-mouthed bottle of such size that the sample completely fills it.

(2) If the sample is more than 200 g, crush it to pass a ½-inch sieve, mix, quarter down to about 200 g, and treat as described in (1).

2 PROCEDURE

Place 50 g of the freshly-powdered sample in a 300 cc beaker, add 150 cc of toluol free from $H_2O$ and non-volatile residue, and dissolve the sample with the aid of heat and occasional shaking. When the soln is apparently complete (no particles of rosin visible), filter at once thru a 25 cc porcelain Gooch crucible which has been previously prepared with a mat of pure, well-washed asbestos (such as is used for the determination of $BaSO_4$) and which has been finally washed thoroly with the solvent used, dried in a boiling-water oven for 30 min., cooled in a desiccator, and weighed. If the rosin filtrate is not clear, return it thru the Gooch crucible until it is clear, finally washing the residue and the outside of the crucible free from rosin with additional hot solvent. Dry the crucible and contents to constant weight at 105–110° in an oven (1 hour usually suffices), cool in a desiccator, weigh, and calculate the percentage of toluol insoluble.

## TURPENTINE OIL[2] (SPIRITS OF TURPENTINE)

3 COLOR—TENTATIVE

Place a 200 cc flat-bottomed colorimeter tube graduated in mm and filled to a depth of 40–50 mm with the turpentine in a colorimeter and on or under it place a No. 2 yellow Lovibond glass. Over or under a second graduated tube in the colorimeter, place a No. 1 yellow Lovibond glass and run into it the same turpentine until the color matches as nearly as possible the color in the first tube. Read the difference in depth of the turpentine in the 2 tubes. If this difference is 50 mm or more, the turpentine is "standard"; if it is 150 mm or more, the turpentine is "waterwhite"; and if the difference is from 25 to 49.9 mm, the turpentine is "off one shade."

4 SPECIFIC GRAVITY

Determine the specific gravity at 15.5/15.5° by any convenient method that is accurate within 2 points in the fourth place. If the determination is made at any other temp., correct the reading by adding thereto or subtracting therefrom 0.00082 for each degree that the temp. at which the determination is made is respectively above or below 15.5°.

5 REFRACTIVE INDEX—TENTATIVE

Determine the refractive index at any convenient temp., but preferably at 20°. If determined at other than 20°, calculate the result to 20° by adding or subtracting the correction factor 0.00045 for each degree that the temp. of the determination is above or below 20°, respectively.

## DISTILLATION—TENTATIVE

### 6 APPARATUS

(a) *Flask.*—Use an Engler flask having the following dimensions: Diameter of bulb, 6.5 cm; cylindrical neck, 15 cm long, 1.6 cm internal diameter; side or vapor tube, 10 cm long, 0.6 cm external diameter, attached to neck at an angle of 75°, so that when the flask contains its charge of 100 cc of oil the surface of the oil shall be 9 cm below the bottom of the junction of the side tube and neck.

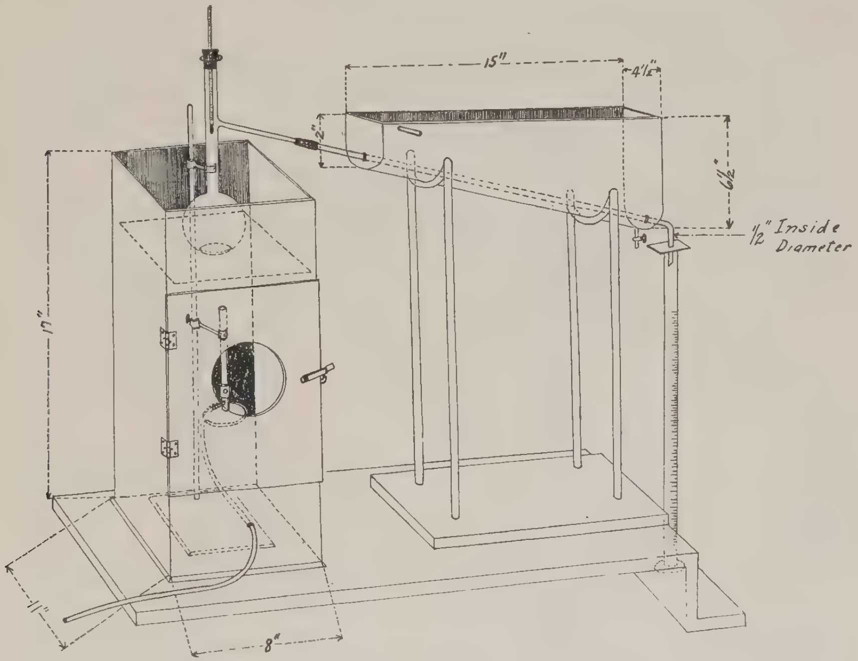

FIG. 9.—APPARATUS FOR THE DISTILLATION OF TURPENTINE OIL OVER OPEN FLAME

Support the flask on a plate of asbestos 20 cm square, having an opening 4 cm in diameter in its center, and heat with an open flame; or, support the flask in a metal cup, 15–20 cm in diameter, containing high boiling mineral oil or gylcerol and fitted with a concave cover having in the center a circular opening 5½–6 cm in diameter (Fig. 10). Surround the flask and burner with a shield to prevent fluctuation in temp. in the neck of the flask.

(b) *Condenser.*—(1) Use the form[3] illustrated in Fig. 9, which consists of thin-walled brass condenser tubing (No. 20 Stubbs gage seamless) ½ inch inside diameter and 22 inches long, placed at an angle of 75° in a metal cooling bath of the size and dimensions shown in Fig. 9. The lower end of the condenser is cut off at an acute angle and curved down for a length of 3 inches so as to project at least ½ inch into the receiving cylinder; or, (2) use a straight glass condenser 22 inches long, having 16 inches in contact with the cooling water and fitted with an adapter, the small end of which, cut off at an acute angle, is long enough to extend a short distance into the receiving cylinder as illustrated in Fig. 10.

(c) *Thermometer.*—Use an accurate thermometer of the Anschutz type, conforming to the following specifications: Graduated from 145 to 200° in 0.2° intervals. Length, bottom of thermometer to 175° mark, not more than 8 nor less than 6.5 cm; top of bulb to 145° mark, not less than 1.5 cm; from 145 to 175° mark, not more than 6 cm. The graduation marks and the numbering shall be clear-cut and distinct. The error at any point on the scale shall not exceed ±0.5° when tested for total immersion of the Hg column.

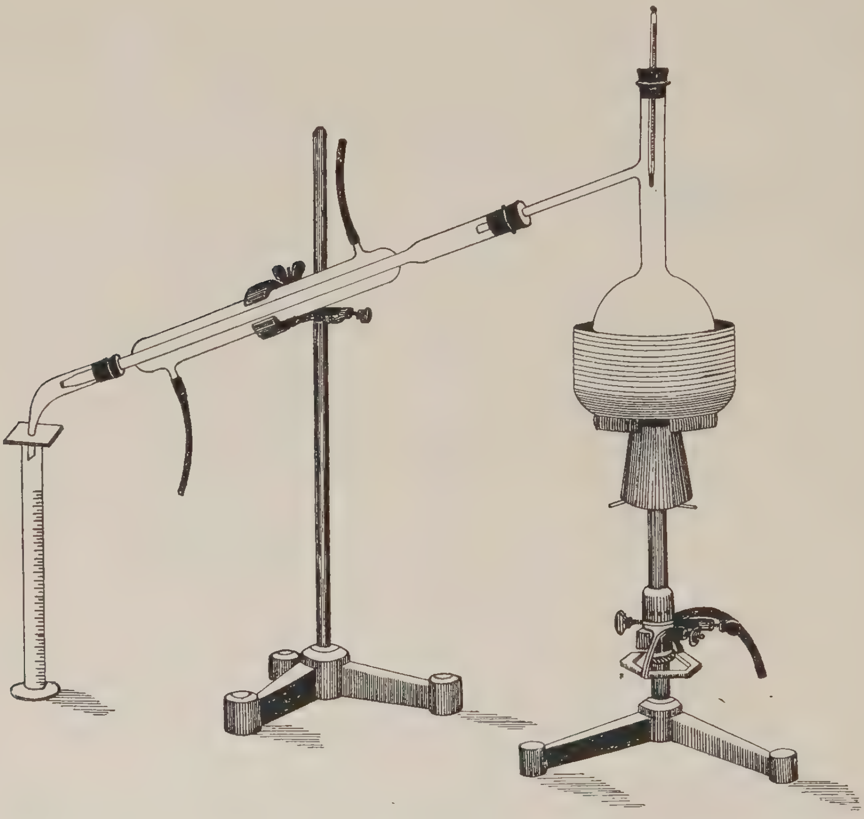

FIG. 10.—APPARATUS FOR THE DISTILLATION OF TURPENTINE OIL OVER BATH

(d) *Receiving cylinder.*—Use an accurately graduated 50–100 cc cylinder. The so-called normal or precision cylinder of 50 cc capacity, having an internal diameter of 1.5 cm and graduated in 0.2 cc is preferred. If a cylinder with larger inside diameter is used, place over the top a pasteboard cover having an opening for the condenser tube.

7 DETERMINATION

Place 100 cc of the turpentine and several small pieces of pumice (or glass) in the distilling flask. Fit the thermometer so that the top of the Hg bulb is level with the bottom of the side tube and the 175° mark is below the cork. Place the flask in position on the asbestos board or oil bath and connect with the condenser. Apply the heat cautiously at first, and when distillation begins so regulate that the turpentine distils at the rate of not less than 4 nor more than 5 cc per min. (approximately 2 drops per second). The initial boiling point is the thermometer reading at the instant when the first drop falls from the end of the condenser. Discontinue distillation when the temp. reaches 170.0°, or the equivalent thereof, depending on the atmospheric pressure, as determined under 8; let the condenser drain; and read the percentage distilled. The percentage distilled below successive selected temps. and the temp. at which each successive 10 cc distils may also be determined, if desired, the necessary correction of the temp. being made for variations in atmospheric pressure.

### 8 CORRECTION FOR VARIATION IN ATMOSPHERIC PRESSURE

The distilling temp. of turpentine is affected 0.057° for each millimeter variation in barometric pressure. If the barometer reading after correcting to 0° is above or below the normal 760 mm, the turpentine will distil at a higher or lower temp., respectively, than at normal pressure. Therefore, for each mm that the corrected barometer reading is above 760 mm, correct the initial boiling-point reading by minus (−) 0.057°; and for each mm that the corrected barometer reading is below 760 mm, correct the initial boiling-point reading by plus (+) 0.057°. Also correct the final temp. observation point (170°) in the same way, by adding thereto 0.057° for each mm of pressure above 760 mm, or subtracting therefrom 0.057° for each mm of pressure below 760 mm, as may be required. The actual temp. at which distillation is stopped must be that equivalent to 170° at 760 mm.

## MINERAL OIL IN TURPENTINE—OFFICIAL

### *Fuming Sulfuric Acid Method*[4]

### 9 REAGENTS

*Fuming 38 N sulfuric acid.*—Mix concentrated sulfuric acid with sufficient liquid fuming $H_2SO_4$ to obtain a mixture containing slightly more than 82.38% total $SO_3$. If the fuming acid contains 50% excess $SO_3$, about 100 g of fuming acid to 140 g of concentrated acid will be approximately the correct ratio. Determine the exact strength of the mixture and also of a reserve supply of concentrated acid as follows:

Weigh a quantity of the acid in a weighing bulb or pipet having a capillary tube at the lower end and a stopcock at the upper end and fitted with a Pt wire for suspending on the balance. Fill the bulb by slight suction and empty the lower end of the capillary by closing the stopcock simultaneously with the withdrawal of the capillary from the acid, wiping off first with a moist and then with a dry cloth. Allow the acid to flow down the sides of the neck of a volumetric flask into cold $H_2O$. (If a flask approximately 100 times the volume of the weighing pipet is used, the resultant soln will be near half-normal). Wash all traces of acid into the flask, taking precautions to prevent loss of $SO_3$ fumes. Make to volume and titrate from a buret against standard alkali, using the indicator with which the alkali was standardized. Calculate the $SO_3$ content of both acids and add sufficient concentrated acid to the fuming mixture to bring it to 82.38%. After mixing, determine the strength of this fuming mixture as before. The $SO_3$ content of this acid must not vary more than +0.05% or −0.08% from 82.38%. The acid must be carefully protected against absorption of moisture from the air.

### 10 DETERMINATION

Place 20 cc of the 38 $N$ fuming $H_2SO_4$ in a graduated narrow-necked Babcock flask, stopper, and place in ice $H_2O$ to cool. Add slowly from a pipet, 5 cc of the turpentine, gently shaking or rotating the flask and keeping the temp. at about 60–65° by continued immersion in ice $H_2O$. When the mixture no longer develops heat on shaking, agitate thoroly by vigorously shaking for about ½ min. Place the flask in a water bath and heat at 60–65° for 10 min., keeping the contents of the flask thoroly mixed by shaking vigorously not less than 6 times during the heating period. (CAUTION: If the shaking is too vigorous at first, there is danger of the escaping $SO_2$ forcing some of the mixture up over the mouth of the flask.) Cool to room temp. and fill the flask with concentrated $H_2SO_4$ until the surface rises well into the graduated neck. Centrifugalize for 5 min. at 1200 r.p.m., or for 10 min. at 900 r.p.m.; or allow to stand, lightly stoppered, for 12 hours. Read the volume of unpolymerized residue (middle of meniscus), calculate the percentage, record its consistency and color, and determine its refractive index at 20°.

Pure gum spirits of turpentine by this method gives less than 2.0% residue, which has a straw or darker color, viscous consistency, and a refractive index of not less than 1.500. A limpid colorless residue with a refractive index of less than 1,500 indicates the presence of mineral oil. The unpolymerized residue from an adulterated oil represents from 60–80% of the total quantity of adulterant present.

### 11 *Sulfuric-Fuming Nitric Acid Method*[5]*—Tentative*

Place 50 cc of the turpentine in a 300 cc Kjeldahl or other long-necked flask, cool in ice $H_2O$, and add slowly with constant agitation 25 cc of $H_2SO_4$. Shake well to obtain complete reaction, keeping the flask cool. When the reaction is complete, cool thoroly and add 25 cc of $H_2O$. Distil the polymerized mixture in a current of steam, collecting 300 cc of total distillate. Separate the oil from the aqueous portions.

Place a volume of fuming (sp. gr. 1.5) $HNO_3$ equal to 3 times the volume of the oil in a 200–250 cc separatory funnel and cool in ice $H_2O$. Add the oil cautiously dropwise, shaking carefully and keeping the mixture cool. After all the oil has been added, allow the funnel to stand quietly, very lightly stoppered, about 30 seconds, until the oil has had a chance to come to the surface. Then draw off the acid and wash the remaining oil once with a little fuming $HNO_3$, once with $HNO_3$, and finally several times with $H_2O$. Measure the volume of the oil, record its consistency and color, and determine its refractive index at 20°. Pure gum spirits of turpentine gives less than 0.5% residue by this method.

## SELECTED REFERENCES

[1] J. Assoc. Official Agr. Chem., **13**, 48 (1930)

[2] U. S. Dept. Agr. Bur. Chem. Bull. **898**; U. S. Bur. Standards Circ., **86**.

[3] Adopted by American Society for Testing Materials.

[4] Chem. Ztg., **30**, 631 (1906); U. S. Dept. Agr. Bur. Chem. Circ., **85**; J. Assoc. Official Agr. Chem., **6**, 465 (1923).

[5] J. Ind. Eng. Chem., 1909, **1**, 27 (1909); J. Assoc. Official Agr. Chem., **5**, 547 (1922); **9**, 55 (1926).

# IX. PAINTS—TENTATIVE

## WHITE LINSEED OIL PAINTS[1]

### 1 PRELIMINARY PROCEDURE

On receipt of a sample make a record of the label, noting especially the brand, the name of the manufacturer, and any statement as to composition and net contents. Weigh the unbroken package, open, note odor and condition of the contents, pour into a clean container, and mix thoroly by pouring from one container to the other, finally leaving the well-mixed sample in the second container, which shall be tighly closed. The well-mixed sample is used at once for the determinations described under "Methods." The original can and cover may be cleaned with gasoline, wiped dry, and then weighed. This weight subtracted from the original weight will give the net weight of the contents. If desired, the specific gravity of the paint may be determined and the weight per gallon calculated, and the volume of paint and the capacity of the container may be measured.

### 2 REAGENTS

(a) *Extraction mixture.*—10 volumes ethyl ether, 6 volumes benzol, 4 volumes methyl alcohol, and 1 volume acetone.

(b) *Aqueous sodium hydroxide.*—Dissolve 100 g of NaOH and dilute to 300 cc.

(c) *Alcoholic sodium hydroxide soln.*—Dissolve NaOH in 95% ethyl alcohol in the proportion of about 22 g per 1000 cc. Let stand in a stoppered bottle. Decant the clear liquid into another bottle and keep well stoppered. This soln should be colorless or only slightly yellow when used; it will keep colorless longer if the alcohol is previously treated with NaOH (about 80 g to 1000 cc) kept at about 50° for 15 days, and then distilled.

(d) *Wijs soln.*—Dissolve I in glacial acetic acid that has a melting point of 14.7–15° and is free from reducing impurities in a proportion that 13 g of I will be present in 1000 cc of soln. The preparation of the I monochloride soln presents no great difficulty but it should be done with care and accuracy in order to obtain satisfactory results. There should be in the soln no sensible excess either of I or more particularly of Cl over that required to form the monochloride. This condition is most satisfactorily attained by dissolving in the whole of the acetic acid to be used the requisite quantity of I, using a gentle heat to assist the soln, if it is found necessary. Set aside a small portion of this soln while pure, and pass dry Cl into the remainder until the halogen content of the soln is doubled. Ordinarily, it will be found that by passing the Cl into the main part of the soln until the characteristic color of free I has just been discharged, there will be a slight excess of Cl which is corrected by the addition of the requisite amount of the unchlorinated portion until all free Cl has been destroyed. A slight excess of I does little or no harm, but excess of Cl must be avoided.

(e) *Standard sodium thiosulfate soln.*—Dissolve pure $Na_2S_2O_3$ in distilled $H_2O$ (well boiled to free it from $CO_2$) in the proportion of 24.83 g of $Na_2S_2O_3.5H_2O$ to 1000 cc of the soln. It is best to let this soln stand for about 2 weeks before standardizing. Standardize with pure resublimed I, K-bi-iodate, or pure $KIO_3$ (see Treadwell-Hall,

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[1] Methods (D 215–29) of the American Society for Testing Materials and adopted tentatively at the 1930 meeting of the A. O. A. C. These methods have been edited to conform in part to the style of this publication, but otherwise they are as published in the 1929 Supplement to Book of A. S. T. M. Standards. Under the standardization procedure of the A. S. T. M., these methods are under the jurisdiction of the A. S. T. M. Committee D-1 on Preservative Coatings for Structural Materials.

Analytical Chemistry, Vol. 2). This soln will be approximately 0.1 *N*, and it is best to leave it as it is after determining its exact I value, rather than to attempt to adjust it to exactly 0.1 *N*. Preserve in a stock bottle provided with a guard tube filled with soda lime.

(f) *Starch soln.*—Stir up 2–3 g of potato starch or 5 g of soluble starch with 100 cc of 1% salicylic acid soln, add 300–400 cc of boiling $H_2O$, and boil the mixture until the starch is practically dissolved, then dilute to 1 liter.

(g) *Potassium iodide soln.*—Dissolve 150 g of KI free from iodate in $H_2O$ and dilute to 1000 cc.

(h) *Acid ammonium acetate soln.*—Mix 150 cc of 80% acetic acid, 100 cc of $H_2O$, and 95 cc of $NH_4OH$ (sp. gr. 0.90).

(i) *Ammonium polysulfide.*—Pass $H_2S$ gas into 200 cc of $NH_4OH$ in a bottle immersed in running $H_2O$ or in iced $H_2O$ until the gas is no longer absorbed; then add 200 cc of $NH_4OH$ and dilute with $H_2O$ to 1000 cc. Digest this soln with 25 g of flowers of S for several hours and filter.

(j) *"Lead acid."*—Mix 300 cc of $H_2SO$ and 1800 cc of $H_2O$. Dissolve 1 g of c. p. Pb-acetate in 300 cc of $H_2O$ and add this to the hot soln, stirring meanwhile. Let stand at least 24 hours and siphon thru a thick asbestos filter.

(k) *Potassium permanganate soln.*—Dissolve 3.2 g of $KMnO_4$ in 1 liter of $H_2O$, let stand 8–14 days, siphon off the clear soln (or filter thru an asbestos filter), and standardize as follows: In a 400 cc beaker dissolve 0.25–0.30 g (accurately weighed) of Bureau of Standards' Na-oxalate in 250 cc of hot $H_2O$ (80–90°) and add 15 cc of sulfuric acid (1+1). Titrate at once with the $KMnO_4$ soln, stirring vigorously and continuously. The $KMnO_4$ must not be added more rapidly than 10–15 cc per min., and the last 0.5–1 cc must be added dropwise with particular care to allow each drop to be fully decolorized before the next is introduced. The temp. of the soln should not be below 60° by the time the end point is reached. (Too rapid cooling may be prevented by allowing the beaker to stand on a small asbestos-covered hot plate during the titration. The use of a small thermometer as a stirring rod is most convenient.) The weight of Na-oxalate used multiplied by 0.833 gives its Fe equivalent. Keep the $KMnO_4$ soln in a glass-stoppered bottle painted black to keep out light.

The Fe value of the $KMnO_4$ multiplied by 1.076 theoretically equals its Sb equivalent. However, for use in determining Sb, the $KMnO_4$ is best standardized as follows: To 0.25 g of pure metallic Sb in a 500 cc Pyrex Erlenmeyer flask, add 12–15 cc of $H_2SO_4$ and 10–12 g of $K_2SO_4$; heat until all the Sb is dissolved, cool, dilute to 250 cc with $H_2O$, add 20 cc of HCl, cool to 10–15°, and titrate with the $KMnO_4$ soln until a faint pink color is obtained. For special work, after digesting, dilute to 100 cc with $H_2O$, add 1–2 g of $Na_2SO_3$, and boil until all the $SO_2$ is expelled. This is shown when no blue color is obtained with starch-iodate paper (r); the volume will be reduced about one-half. Dilute to 250 cc with $H_2O$, add 20 cc of HCl (sp. gr. 1.19), and complete as described.

(l) *Standard potassium ferrocyanide.*—Dissolve 22 g of the pure salt in $H_2O$ and dilute to 1000 cc. To standardize, transfer about 0.2 g (accurately weighed) of pure metallic Zn or freshly ignited pure ZnO to a 400 cc beaker. Dissolve in 10 cc of HCl and 20 cc of $H_2O$. Drop in a small piece of litmus paper, add $NH_4OH$ until slightly alkaline, then add HCl until just acid, and then 3 cc of HCl. Dilute to about 250 cc with hot $H_2O$ and heat nearly to boiling. Run in the ferrocyanide soln slowly from a buret with constant stirring until a drop tested on a white porcelain plate with a drop of the uranyl indicator shows a brown tinge after standing 1 min. A blank should be run with the same amounts of reagents and $H_2O$ as in the standardization. The amount of ferrocyanide soln required for the blank should be subtracted from the amounts used in standardization and in titration of the sample. The standardization must be made under the same conditions of temp., volume, and acidity as obtained when the sample is titrated.

(m) *Uranyl indicator for zinc titration.*—A 5% soln of uranyl nitrate in $H_2O$ or a 5% soln of uranyl acetate in $H_2O$ made slightly acid with acetic acid.

(n) *Alkaline lead nitrate soln.*—Into 100 cc of KOH soln (56 g in 140 cc of $H_2O$) pour a saturated soln of $Pb(NO_3)_2$ (250 g in 500 cc of $H_2O$) until the precipitate ceases to redissolve, stirring constantly while mixing. Let settle, filter thru asbestos, and dilute the clear filtrate with an equal volume of $H_2O$. About 3 volumes of the $Pb(NO_3)_2$ soln will be required for 1 of the KOH.

(o) *Ammoniacal cadmium chloride or zinc sulfate soln.*—Dissolve 8 g of $CdCl_2$ in 200 cc of $H_2O$ and add 200 cc of $NH_4OH$ (sp. gr. 0.90), or dissolve 50 g of $Zn(SO_4)_2$ in 270 cc of $H_2O$ and 230 cc of $NH_4OH$ (sp. gr. 0.90).

(p) *Standard potassium iodate soln.*—Dissolve 3.6 g of $KIO_3$ and 39 g of KI in 1000 cc of $H_2O$. For general work the theoretical sulfur titer of this soln should be used; for special work, the soln may be standardized against like material, such as a lithopone of known sulfide-S content. The theoretical titer is based on standard $Na_2C_2O_4$ and is obtained as follows: To 300 cc of $H_2O$ in a 600 cc flask, preferably glass-stoppered, add 10 cc of HCl (sp. gr. 1.19) and 1 g of KI. Cool, and add 10 cc of 0.1 $N$ $KMnO_4$ soln which has been standardized against $Na_2C_2O_4$. Swirl gently, stopper, and let stand for 5 min. Titrate the liberated I with standard $Na_2S_2O_3$ soln until the color fades. Then add 10 cc of starch soln and continue the titration until the blue color is destroyed. Repeat the titration, substituting 10 cc of the iodate soln for the $KMnO_4$ soln. Calculate the normality of the iodate soln.

(q) *Starch indicator for sulfur titration.*—(1) To 1000 cc of boiling $H_2O$, add a cold suspension of 6 g of starch in 100 cc of $H_2O$ and boil vigorously for 5 min. Cool the soln, add 6 g of $ZnCl_2$ dissolved in 50 cc of cold $H_2O$, thoroly mix, and set aside for 24 hours. Decant the clear supernatant liquid into a suitable container, add 3 g of KI, and mix thoroly. (2, Optional.) Prepare an emulsion of 6 g of soluble starch in 25 cc of $H_2O$, add a soln of 1 g of NaOH in 10 cc of $H_2O$, and stir the soln until it gelatinizes. Dilute to 1000 cc with $H_2O$, add 3 g of KI, and mix thoroly.

(r) *Starch-iodate paper.*—Impregnate filter paper with a soln obtained by heating 2 g of starch with 100 cc of $H_2O$, and, after soln, adding 0.2 g of $KIO_3$ dissolved in 5 cc of $H_2O$.

(s) *Standard iodine soln for $SO_2$.*—Place 15–20 g of pure KI in a liter volumetric flask, dissolve in as little $H_2O$ as possible, and then add about 6.4 g of resublimed I. Shake until all the I is dissolved, dilute to the mark with $H_2O$, and mix. This soln is approximately 0.05 $N$ and is standardized against 0.05 $N$ $Na_2S_2O_3$ to obtain its true normality.

(t) *Standard sodium thiosulfate soln for $SO_2$.*—Prepare and standardize as described in (e), using 12.42 g of $Na_2S_2O_3 . 5H_2O$; or the 0.1 $N$ soln may be diluted with an equal volume of cold $CO_2$-free $H_2O$.

(u) *Ferric sulfate soln for titanium.*—Prepare a soln containing 2% of Fe as ferric sulfate as follows: Dissolve 20 g of pure Fe or plain C steel in a slight excess of HCl, oxidize with $HNO_3$, add about 80 cc of $H_2SO_4$ and heat until fumes of the latter are evolved. Cool, dilute with $H_2O$ to 1000 cc, digest on a steam bath until sulfates are dissolved, and filter if necessary. Add 0.1 $N$ $KMnO_4$ soln until a faint pink color persists for 5 min. (to oxidize any ferrous Fe that may be present). Ferric ammonium sulfate may be used also.

(v) *Standard ferric sulfate soln for colorimetric determination of iron.*—Determine the strength of the ferric soln for the $TiO_2$ determination in terms of Fe and dilute a portion of this soln until one is obtained of the strength 1 cc = 0.00001 g of Fe.

(**w**) *Potassium thiocyanate indicator.*—Prepare a 2% soln of the pure salt in $H_2O$.

## METHODS

### 3 WATER[1]

Mix 100 g of the paint in a 250 cc flask with 75 cc of toluene. Place the flask in an oil bath, connect with condenser, apply heat to the bath, and distil until about 50 cc of distillate has been collected in a graduate. The temp. in the flask should be then 105–110°. The number of cc of $H_2O$ collected under the toluene in the receiver is the percentage of $H_2O$ in the paint.

### 4 VOLATILE THINNER

Weigh accurately 3–5 g of the paint into a tared flat-bottomed dish about 8 cm in diameter, spreading the paint over the bottom. Heat at 105–110° for 1 hour, cool, and weigh. Calculate the loss in weight as percentage of $H_2O$ and volatile thinner, subtract from this the percentage of $H_2O$ (**3**), and report the remainder as volatile thinner.

### 5 NATURE OF THE THINNER

Transfer about 150 g of the paint to a 500 cc flask fitted with a 2-hole cork stopper carrying a spray trap connected with a vertical condenser. Thru the other hole in the stopper pass an influx tube for steam. (This tube should dip below the surface of the paint.) Heat the flask in an oil bath or an air bath at 100° and pass thru it a current of steam; with the steam still passing thru, raise the temperature of the bath to 130°. Catch the distillate in a small separatory funnel and continue distillation until 300 cc of $H_2O$ has been condensed. Portions of this $H_2O$ may be drawn from the cock of the separatory funnel from time to time, but care must be taken not to draw out any of the volatile thinner. Let the distillate stand until it separates into 2 layers, then draw off the $H_2O$, and filter the volatile thinner thru a dry filter paper into a dry flask. If the thinner is apparently turpentine, examine the distillate as directed[2] in Chap. **VIII.** If the thinner is a mixture of turpentine and mineral spirits, an approximate determination of the amount of turpentine may be made by the polymerization test specified for under turpentine. It should be noted that turpentine is slightly soluble in $H_2O$ (about 0.3–0.4 cc per 100 cc of $H_2O$).

To test for benzol, add a few drops of the distillate to a small quantity of a mixture of $HNO_3$ and $H_2SO_4$, and heat cautiously. The characteristic odor of nitrobenzol will be noted if benzol is present.

If the thinner is apparently all mineral spirits, no further examination is necessary.

### 6 PERCENTAGE OF PIGMENT

Strain a portion of the well-mixed sample thru a No. 80 sieve[3] to remove any skins and weigh accurately about 15 g of the strained paint in a weighed centrifuge tube. Add 20–30 cc of "extraction mixture" [Reagent **2(a)**], mix thoroly with a glass rod, wash the rod with more of the extraction mixture, and add enough of the reagent to make a total of 60 cc in the tube. Place the tube in the container of a centrifuge, surround the tube with $H_2O$, and counterbalance the container of the opposite arm with a similar tube, or a tube with $H_2O$. Whirl at a moderate speed until well settled. Decant the clear supernatant liquid, repeating the extraction twice with 40 cc of extraction mixture and once with 40 cc of ethyl ether. After drawing off the ether, set the tube in a beaker of $H_2O$ at about 80° or on top of a warm oven for 10 min., then in an oven at 105–110° for 2 hours. Cool, weigh, and calculate the percentage of pigment. Grind the pigment to a fine powder, pass thru a No. 80 sieve to remove any skins, and preserve in a stoppered bottle.

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[1] A convenient apparatus for this determination is shown in Fig. 1 (*b*) of the Standard Method of Test for Water in Petroleum Products and Other Bituminous Materials (A.S.T.M. Designation: D 95) of the American Society for Testing Materials. (1928 Supplement to Book of A.S.T.M. Standards, p. 149.)

[2] 1927 Book of A.S.T.M. Standards, Part II, p. 269 or 1930 ed., Part II, p. 337.

[3] Sieve opening .177 mm; wire diameter .119 mm.

### 7 PERCENTAGE OF NON-VOLATILE VEHICLE

Add together the percentages of $H_2O$, of volatile thinner, and of pigment, and subtract the sum from 100. Report the remainder as non-volatile vehicle.

## TESTING NON-VOLATILE VEHICLE

### 8 PREPARATION OF FATTY ACIDS

To about 25 g of the paint in a porcelain casserole, add 15 cc of aqueous NaOH [Reagent **2(b)**] and 75 cc of ethyl alcohol, mix, and heat uncovered on a steam bath until all volatile thinner is driven off and saponification is complete. Add 100 cc of $H_2O$, boil, add $H_2SO_4$ (sp. gr. 1.2) (8–10 cc in excess), boil, stir, and transfer to a separatory funnel to which some $H_2O$ has been previously added. Draw off as much as possible of the acid aqueous layer and any insoluble or precipitated matter, wash once with $H_2O$, then add 50 cc of $H_2O$ and 50 cc of ethyl ether. Shake very gently with a whirling action to dissolve the fatty acids in the ether, but not so violently as to form an emulsion. Draw off the aqueous layer and wash the ether layer with one 15 cc portion of $H_2O$ and then with 5 cc portions of $H_2O$ until free from $H_2SO_4$. Then draw off the $H_2O$ layer completely. Transfer the ether soln to a dry flask and add 25–50 g of anhydrous $Na_2SO_4$. Stopper the flask and let stand with occasional shaking at a temp. below 25° until the $H_2O$ is completely removed from the ether soln, which will be shown by the soln becoming perfectly clear above the solid $Na_2SO_4$. Decant this clear soln, if necessary, thru a dry filter paper into a dry 100 cc Erlenmeyer flask. Pass a rapid current of dry air (pass thru a $CaCl_2$ tower) into the mouth of the Erlenmeyer flask and heat to a temp. below 75° on a dry hot plate until the ether is entirely driven off. It is important to follow all the details, as ether generally contains alcohol, and after washing with $H_2O$ always contains $H_2O$. It is difficult to remove $H_2O$ and alcohol by evaporation from fatty acids, but the washing of the ether soln and subsequent drying with anhydrous $Na_2SO_4$ removes both $H_2O$ and alcohol. Ether, in the absence of $H_2O$ and alcohol, is easily removed from fatty acids by gentle heat. If the pigment settles out rapidly in a sample of the paint on standing so that sufficient vehicle can be poured off, or if sufficient vehicle is obtained by centrifugalizing the paint, it will be advantageous to saponify this separated vehicle and liberate and prepare the fatty acids as described.

The above method of preparing the fatty acids directly from the material, rather than from the extracted vehicle, is based upon past experience in sometimes obtaining too low results by the latter method. Occasionally, however, trouble is experienced in saponifying the entire material, due to interference of pigment. In such an instance it is permissible to save the extracted vehicle (see **6**), evaporate the organic solvents on a steam bath, and saponify and prepare the fatty acids in the usual manner from this extract. If the I number obtained in this manner passes a given specification, no further work is necessary; if the I number is low, it will be necessary to repeat the work directly on the entire material.

The fatty acids prepared as above should be kept in a stoppered flask and examined at once.

### 9 TEST FOR MINERAL OIL AND OTHER UNSAPONIFIABLE MATTER

Place 10 drops of the fatty acids (**8**), in a 50 cc test tube, add 5 cc of alcoholic soda [Reagent **2**(**c**)], boil vigorously for 5 min., add 40 cc of $H_2O$, and mix. A clear soln indicates that not more than traces of unsaponifiable matter are present.

### 10 IODINE NUMBER OF FATTY ACIDS[1]

Place a small quantity of the fatty acids (**8**), in a small weighing buret or beaker. Weigh accurately. Transfer by dropping about 0.15 g (0.10–0.20 g) into a 500 cc bottle having a well-ground-glass stopper, or an Erlenmeyer flask having a specially flanged neck for the I test. Reweigh the buret or beaker and determine the amount of sample used. (If desired the sample may be weighed in a small wide-mouthed vial and the vial containing the weighed sample placed in the bottle or flask.) Add 10 cc of $CHCl_3$. Whirl the bottle or flask to dissolve the sample. Add 10 cc of $CHCl_3$ to 2 empty bottles or flasks like that used for the sample. Add to each bottle or flask 25 cc of the Wijs soln [Reagent **2**(**d**)] and let stand with occasional shaking for 1 hour in a dark place at a temp. of from 21 to 23°. Add 10 cc of the 15% KI soln and 100 cc of $H_2O$, and titrate with standard $Na_2S_2O_3$ soln [Reagent **2**(**e**)], using starch as indicator. The titrations on the 2 blank tests should agree within 0.1 cc. From the difference between the average of the blank titrations and the titration on the sample and the I value of the thiosulfate soln, calculate the I number of the sample tested. (I number is given in centigrams of I to 1 g of sample.)

### 11 ROSIN

#### *I. Liebermann-Storch Test*[2]

To about 1 g of the fatty acids add 15 cc of acetic anhydride and shake until soln is complete. Pour a few drops of this soln on a white porcelain plate (a crucible cover serves well) and add a drop of $H_2SO_4$ (sp. gr. 1.53). A fugitive violet color indicates rosin.

#### *II. Halphen-Hicks Test*[3]

Test the fatty acids with the Halphen-Hicks reagent as follows:

*Soln A.*—Dissolve 1 part by volume of phenol in 2 parts by volume of $CCl_4$.

*Soln B.*—Dissolve 1 part by volume of Br in 4 parts by volume of $CCl_4$.

Add 1–2 cc of Soln A to about 1 g of the fatty acids and pour this mixture into a cavity of an ordinary porcelain color-reaction plate until it just fills the depression. Immediately fill an adjacent cavity with Soln B. Cover the plate with an inverted watch-glass and note the color, if any, produced in the former soln by the action of the Br vapors from Soln B. A decided purple or deep indigo blue color is an indication of the presence of rosin.

## PIGMENT

### 12 *A. Qualitative Analysis*

A complete qualitative analysis, following the well-established methods, should be made and the quantitative scheme modified as required. Add acetic acid slowly to the pigment until all carbonate is decomposed (noting whether any $H_2S$ is evolved);

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[1] If appreciable amounts of rosin or of unsaponifiable matter are found to be absent in the vehicle of a paint, the I number of the fatty acids gives the best indication (tho not proof) of the presence of linseed oil. An I number of less than 175 (Wijs) for the fatty acids is an indication that the non-volatile vehicle was not pure linseed oil.

[2] Lewkowitsch, Chemical Technology and Analysis of Oils, Fats and Waxes, Vol. 1, p. 623 (1921).

[3] *J. Ind. Eng. Chem.*, **3**, 86 (1911).

then add a large excess of acid $NH_4$-acetate soln [Reagent **2**(h)], boil, filter, and test the filtrate for metals other than Pb and Zn (especially Ca and Ba). The absence of Ca in this filtrate indicates that the extending pigments contain no $CaCO_3$ or $CaSO_4$; the absence of Ba indicates that the extending pigments contain no $BaCO_3$.[1] Wash the matter insoluble in acid $NH_4$-acetate soln with another portion of this soln, and finally with hot $H_2O$. This insoluble matter is dried, ignited, and tested for siliceous matter, $BaSO_4$, and Ti compounds. To test for the Ti compounds, place a small amount of the insoluble matter, or of the original sample (about 0.5 g), in a 250 cc Pyrex glass beaker; add 20 cc of concentrated $H_2SO_4$ and 7–8 g of $(NH_4)_2SO_4$. Mix well, and boil for a few min. A residue denotes the presence of $SiO_2$, siliceous matter, or $BaSO_4$. Cool the soln, dilute with 100 cc of $H_2O$, heat to boiling, settle, filter, wash with hot 5% $H_2SO_4$ until free from Ti. The residue may be tested for Pb, Ba, and $SiO_2$. Add $H_2O_2$ to a small portion of the filtrate; a clear yellow-orange color indicates the presence of Ti. Boil another portion of the filtrate with metallic tin or Zn. A pale blue to violet coloration indicates Ti. Treat another portion (about 1 g) of the pigment with 20 cc of HCl (1+1) and note whether any $H_2S$ is evolved; boil the soln for about 5 min., add about 25 cc of hot $H_2O$, filter, and wash with hot $H_2O$. Render a small portion of the filtrate alkaline with $NH_4OH$, acidify with HCl, and add a little $BaCl_2$ soln; a white precipitate ($BaSO_4$) indicates the presence of a soluble sulfate. To another portion of the filtrate add a little $H_2SO_4$ (a white precipitate indicates the presence of Pb, soluble Ba or both (some $CaSO_4$ may also separate); filter, wash to remove free acid, and treat the precipitate with a few drops of KI soln (the formation of yellow $PbI_2$ indicates the presence of Pb). The white precipitate may also be treated with $H_2S$ water; the formation of black PbS indicates the presence of lead. To another portion of the original filtrate add $NH_4OH$ until alkaline, render slightly acid with acetic acid, heat to boiling, and add a little $K_2Cr_2O_7$ soln; a yellow or orange-yellow precipitate indicates the presence of Pb, soluble Ba or both. To another portion of the original filtrate add a few drops of $K_4Fe(CN)_6$ soln. A white precipitate with a bluish tinge indicates the presence of Zn. Pass into the remaining portion of the original filtrate a current of $H_2S$ for 5–10 min., add an equal volume of $H_2O$ and pass $H_2S$ into the soln for about 5 min.; filter, wash with $H_2S$ water; then digest the precipitate with $NH_4$-polysulfide, filter, acidify the filtrate with HCl, and warm; the presence of Sb is indicated by the separation of an orange colored precipitate. The filtrate from the $H_2S$ precipitate may be tested for Ba, Ca, and Mg in the usual manner.

### 13 *B. Quantitative Analysis*

### 14 SPECIFIC GRAVITY

If the determination of specific gravity of the pigment is required, determine according to the Standard Methods of Test for Specific Gravity of Pigments (Serial Designation: D 153) of the American Society for Testing Materials.[2]

## SINGLE PIGMENTS

### 15 BASIC CARBONATE OF LEAD

(a) *Total Lead (Gravimetric).*—Dissolve 1 g in 20 cc of $HNO_3$ (1+1) in a covered beaker, heating till all $CO_2$ is expelled; wash off cover, add 20 cc of $H_2SO_4$ (1+1) and evaporate to fumes of $SO_3$, cool, add about 150 cc of $H_2O$ and 150 cc of alcohol; let stand in *cold* $H_2O$ 1 hour, filter on a Gooch crucible, wash with 95% alcohol, dry at 110°, and weigh $PbSO_4$; calculate to PbO or to basic carbonate.[1] Instead of determining the Pb as sulfate, the sample may be dissolved by boiling with acetic acid; then dilute to about 200 cc with $H_2O$, make alkaline with $NH_4OH$, then acid with acetic acid, heat to boiling and add 10–15 cc of a 10% soln of $K_2Cr_2O_7$; heat till the yellow precipitate assumes an orange color. Let settle and filter on a Gooch crucible, washing by decantation with hot $H_2O$ till the washings are colorless, finally transferring all the precipitate. Wash with 95% alcohol and then with ether; dry at 110° and weigh $PbCrO_4$. (Any insoluble matter should be filtered out before precipitating the Pb.)

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[1] If the original sample contained $BaCO_3$ and $PbSO_4$, $CaSO_4$ or other soluble sulfate, the soluble Ba will form with the soluble sulfate a precipitate of $BaSO_4$, which will be determined as "insoluble matter." If the sample contained $SrSO_4$ or $SrCO_3$, some $SrSO_4$ may be counted as $BaSO_4$, some Sr will count as soluble Ba, and some may be counted as CaO. This element is not separated, as it probably will not be encountered, or will be present as an impurity in the Ba and Ca compounds.

[2] 1927 A. S.T.M. Standards, Vol. II, p. 277 or 1930 A.S.T.M. Standards, Part, p. 345.

(b) *Total Lead (Volumetric).*—Dissolve 0.5 g of sample in 10 cc of HCl, boil till soln is effected, cool, dilute to 40 cc, neutralize with $NH_4OH$. Add acetic acid until distintly acid, dilute to 200 cc with hot $H_2O$, boil and titrate with $NH_4$-molybdate as follows:

Dissolve 4.25 g of $NH_4$-molybdate in $H_2O$ and make up to 1 liter. To standardize this soln, dissolve about 0.2 g of pure Pb-foil in $HNO_3$ (pure PbO or $PbSO_4$ may also be used), evaporate nearly to dryness, add 30 cc of $H_2O$, then 5 cc $H_2SO_4$ (sp. gr. 1.84), cool, and filter. Drop filter with $PbSO_4$ into a flask, add 10 cc of HCl, boil till completely disintegrated, add 15 cc of HCl, 25 cc of $H_2O$, and $NH_4OH$ till alkaline. Acidify with acetic acid, dilute to 200 cc with hot $H_2O$ and boil. Titrate, using an outside indicator of 1 part of tannic acid in 300 parts of $H_2O$.

It should be noted that when Ca is present, it forms a more or less insoluble molybdate, and results are apt to be high. With samples containing less than 10% of Pb, the Pb should be precipitated as $PbSO_4$, filtered, redissolved and titrated as in the process of standardizing.

(c) *Lead Carbonate and Lead Hydroxide.*—Determine $CO_2$ by evolution with dilute HCl, absorbing in soda lime or KOH soln. Calculate $CO_2$ to $PbCO_3$, subtract PbO equivalent from total PbO and calculate residual PbO to $Pb(OH)_2$.

The following method of A. N. Finn (unpublished) gives total basicity of a pure white lead. Place 2 g of pigment in an evolution flask, add a little $CO_2$-free $H_2O$, connect up to the separatory funnel and condenser (Knorr type), add thru the funnel, finally washing down, 100 cc of 0.25 $N$ $HNO_3$, boil, and absorb the $CO_2$ in soda-lime tube in usual manner (having $H_2SO_4$ and $CaCl_2$ drying tubes in train) and weigh. To the soln in the evolution flask add about 20 cc of neutral $Na_2SO_4$ soln and titrate with 0.25 $N$ NaOH soln (carbonate-free), using phenolphthalein. $CO_2$ is calculated to $PbCO_3$. The amount of 0.25 $N$ acid corresponding to the $CO_2$ is calculated and deducted from the total amount of 0.25 $N$ acid neutralized by the sample and the difference is calculated to $Pb(OH)_2$.

## 16 BASIC SULFATE OF LEAD[2]

*Total Lead.*—Dissolve 1 g of the sample in 100 cc of a mixture consisting of 125 cc of 80% acetic acid, 95 cc of $NH_4OH$ (sp. gr. 0.90) and 100 cc of $H_2O$. Add the soln while hot, dilute with about 50 cc of $H_2O$ and boil until sample is dissolved. Dilute to 200 cc and titrate with standard $NH_4$-molybdate soln as described under basic carbonate of Pb. The $NH_4$-molybdate soln contains about 8.67 g per liter and is standardized against pure Pb-foil, pure PbO or pure $PbSO_4$.

*Total Zinc.*—Boil 1 g of the sample with 30 cc of $H_2O$, 4 g of $NH_4Cl$ and 6 cc of HCl (some $PbSO_4$ or $PbCl_2$ may not dissolve). Dilute to 200 cc with hot $H_2O$, add

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[1] This method of weighing lead sulfate is not accurate in the presence of calcium compounds.

[2] J. A. Schaeffer's method, *J. Ind. Eng. Chem.*, **6**, 200 (1914), may be used.

2 cc of a saturated soln of $Na_2S_2O_3$ and titrate with a standard soln of $K_4Fe(CN)_6$ in usual manner. Calculate the Zn to ZnO.[1]

99.70[2] − percentage of ZnO found = percentage of Pb constituents; then

$$\frac{\left(\frac{\text{Molecular weight PbSO}_4}{\text{Atomic weight Pb}} \times \begin{matrix}\text{Percentage of}\\ \text{Pb found}\end{matrix}\right) - \begin{matrix}\text{Percentage of}\\ \text{Pb constituents}\end{matrix}}{\frac{\text{Molecular weight PbSO}_4 - \text{Molecular weight PbO}}{\text{Molecular weight PbO}}} = \begin{matrix}\text{percentage of}\\ \text{PbO}\\ \text{present.}\end{matrix}$$

$$\frac{\left(\frac{\text{Molecular weight PbO}}{\text{Atomic weight Pb}} \times \begin{matrix}\text{Percentage of}\\ \text{Pb found}\end{matrix}\right) - \begin{matrix}\text{Percentage of}\\ \text{Pb constituents}\end{matrix}}{\frac{\text{Molecular weight PbO} - \text{Molecular weight PbSO}_4}{\text{Molecular weight PbSO}_4}} = \begin{matrix}\text{percentage}\\ \text{of PbSO}_4\\ \text{present.}\end{matrix}$$

## 17 ZINC OXIDE

*Total Zinc.*—Dissolve 0.25 to 0.3 g in 10 cc of HCl and 20 cc of $H_2O$; make alkaline with $NH_4OH$, then acid with HCl; add 3 cc more of HCl, dilute to about 250 cc with $H_2O$, heat nearly to boiling, and titrate with standard $K_4Fe(CN)_6$ soln as described by Low.[3] Report as ZnO (includes Cd). Fe, Cu, or other interfering substances should be first removed as described by Low.

*Total Soluble Sulfur.*[4]—Moisten a 10 g sample with $H_2O$, add a few drops of Br and then HCl, boil to expel Br, filter from any insoluble, and wash with hot $H_2O$. Make alkaline with $NH_4OH$, then just slightly acid with HCl, heat to boiling, and add about 15 cc of hot $BaCl_2$ soln. Let stand several hours (overnight), filter on a weighed Gooch crucible, wash well with hot $H_2O$, dry, ignite for 5 min., cool, and weigh as $BaSO_4$. Calculate to S.

## 18 LITHOPONE

(Ponolith, Jersey Lily White, Becton White, Charlton White, Orr's White)

*Insoluble and Total Zinc.*—Take 1 g of the sample in a 200 cc beaker, add 10 cc of HCl, mix, and add in small portions about 1 g of $KClO_3$, then heat on the steam bath until about half of the liquid is evaporated. Dilute with $H_2O$, add 5 cc of $H_2SO_4$ (1+10); boil, let settle, filter, wash, ignite, cool, and weigh the insoluble which should be only $BaSO_4$; make a qualitative examination for $Al_2O_3$ and $SiO_2$. The insoluble should be examined under the microscope for the presence of natural crystalline barytes. Sample may also be examined direct. Make filtrate from insoluble alkaline with $NH_4OH$, acid with HCl, add 3 cc of HCl, dilute to about 250 cc with $H_2O$, heat nearly to boiling, and titrate with $K_4Fe(CN)_6$ soln as under zinc white. Calculate to Zn.

*Zinc Oxide.*—Treat a 4 g sample of the lithopone for 4 hours with 200 cc of 1% acetic acid at room temp., stirring occasionally. Filter by suction on a double filter paper and wash with cold $H_2O$; add to the clear filtrate 13 cc of $NH_4OH$, neutralize with HCl, and then add 3 cc of HCl in excess. Heat to boiling and titrate with $K_4Fe(CN)_6$, using uranium-acetate soln as an outside indicator. Calculate to ZnO.

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[1] It would probably be more accurate to remove total Pb as $PbSO_4$, and titrate the Zn in the filtrate after adding $NH_4OH$ and HCl in usual manner.

[2] This figure (99.70) is the average total percentage of $PbSO_4$, PbO and ZnO, found in 270 total analyses of basic lead sulfate.

[3] Low, Technical Methods of Ore Analysis.

[4] Method of G. Rigg.

Calculate this result to Zn, subtract from total Zn, and calculate the difference to ZnS. (Any $ZnCO_3$ or $ZnSO_4$ is included in the ZnO.)

*Zinc Sulfide.*[1]—Place 0.5 g of pigment in evolution flask with about 10 g of "feathered" or mossy Zn, add 50 cc of $H_2O$; insert the stopper carrying a separatory funnel and an exit tube. Run in 50 cc of HCl from the funnel, having previously connected the exit tube to 2 absorption flasks in series; first flask contains 100 cc of alkaline $Pb(NO_3)_2$ soln, second flask, 50 cc of same as a safety device. After all the acid has run into the evolution flask, heat slowly, finally boiling until the first appearance of steam in the first absorption flask; disconnect, let the PbS settle, filter, wash with cold $H_2O$, then with hot $H_2O$ till neutral to litmus paper and washings give no test for Pb. The PbS precipitate is dissolved in hot, dilute $HNO_3$, evaporated to fumes with $H_2SO_4$ and finally weighed as $PbSO_4$. Calculate PbS or $PbSO_4$ to ZnS.

The alkaline Pb-soln is made as follows: Into 100 cc of KOH soln (56 g in 140 cc of $H_2O$) pour a saturated soln of $Pb(NO_3)_2$ (250 g in 500 cc of $H_2O$) until the precipitate ceases to redissolve, stirring constantly while mixing. About 3 volumes of the Pb soln will be required for 1 of the alkali.

Instead of absorbing the evolved $H_2S$ in alkaline $Pb(NO_3)_2$ soln a soln of 8 g of $CdCl_2$ in 250 cc of $H_2O$ and 150 cc of $NH_4OH$ (sp. gr. 0.90) may be used. The CdS precipitate may be filtered on a weighed Gooch, washed with $H_2O$ containing a little $NH_4OH$, dried at 100°, and weighed. Calculate to ZnS. It is better to filter the CdS on a small filter and wash as above, then place filter and precipitate in a beaker and dissolve in HCl and $KClO_3$ (keeping at room temp. at first), filter out any paper pulp or insoluble matter; make filtrate alkaline with $NH_4OH$, then just acid with HCl, heat to boiling and precipitate with $BaCl_2$ in usual manner. Filter, wash, ignite, and weigh $BaSO_4$. Calculate to ZnS.

For very rapid work the contents of the absorption flask, after all $H_2S$ has been absorbed, may be washed into a vessel with *cold* $H_2O$ and diluted to about 1 liter, acidified with HCl and titrated with standard I soln. Use starch indicator. (The precipitate should be completely dissolved.) The I soln is prepared by dissolving about 12.7 g of pure resublimed I and 18 g of KI in a little $H_2O$ and then diluting to 1 liter.

### 19 TITANIUM PIGMENT

*Titanium oxide.*—Transfer 0.5 g of the dried sample to a 250-cc Pyrex beaker, add 20 cc of $H_2SO_4$ and 7–8 g of $(NH_4)_2SO_4$. Mix well and heat on hot plate until fumes of $H_2SO_4$ are evolved, and then continue the heating over a strong flame until soln is complete (usually requires not over 5 min. of boiling) or it is apparent that the residue is composed of $SiO_2$, siliceous matter, or $BaSO_4$. Caution should be observed in visually examining this hot soln. Cool the soln, dilute with 100 cc of $H_2O$, stir, heat carefully to boiling while stirring, let settle, filter thru paper, and transfer the precipitate completely to the paper. Wash the insoluble residue with cold 5% (by volume) $H_2SO_4$ until Ti is removed.

Dilute the filtrate to 200 cc and add about 10 cc of $NH_4OH$ (sp. gr. 0.90) to lower the acidity to approximately 5% $H_2SO_4$ (by volume).

Wash out a Jones reductor[2] with dilute 5% by volume $H_2SO_4$ and $H_2O$, leaving sufficient $H_2O$ in the reductor to fill to the upper level of the Zn. (These washings should require not more than 1 or 2 drops of 0.1 *N* $KMnO_4$ soln to obtain a pink color.) Empty the receiver, and put in it 25 cc (measured in a graduate) of ferric sulfate soln [Reagent **2**(u)]. Reduce the prepared Ti soln as follows:[3]

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[1] Evolution method of W. G. Scott, White Paints and Painting Material, p. 257; see also Blair, Chemical Analysis of Iron.

[2] Directions for preparing a Jones reductor may be found in Blair. The Chemical Analysis of Iron, 8th Ed., pp. 88–89, or Treadwell-Hall, Analytical Chemistry, Vol. 2, 5th Ed.

[3] Lundell and Knowles, The Determination of Titanium by reduction with Zinc and Titration with Permanganate, *J. Am. Chem. Soc.*, **45**, 2620 (1923).

(1) Run 50 cc of the 5% $H_2SO_4$ soln thru the reductor at a speed of about 100 cc per min.

(2) Follow this with the Ti soln.

(3) Wash out with 100 cc of 5% $H_2SO_4$.

(4) Finally run thru about 100 cc of $H_2O$. Care should be taken that the reductor is always filled with soln or $H_2O$ to the upper level of the Zn. Gradually release the suction, wash thoroly the glass tube that was immersed in the ferric sulfate soln, remove the receiver, and titrate immediately with 0.1 *N* $KMnO_4$ soln. 1 cc of 0.1 *N* $KMnO_4 = 0.0048$ g of Ti or 0.008 g of $TiO_2$. Run a blank determination, using the same reagents, washing the reductor as in the above determination. Subtract this permanganate reading from the original reading and calculate the final reading to $TiO_2$. This will include Fe, Cr, As, and any other substance which is reduced by Zn and acid. See calculations under **21** for reporting $TiO_2$.

**20**

*Iron oxide.*—Weigh a 1-g portion of the sample and treat as in **19**. Transfer without filtering to a graduated 200 cc flask, cool, fill to the mark with $H_2O$, mix, let settle, and determine Fe colorimetrically as follows: Filter thru a dry filter paper, discarding the first 20 cc; transfer 50 cc of the clear filtrate to a clean 100 cc Nessler tube or other comparator. Add a drop or two of 0.1 *N* $KMnO_4$ soln to oxidize any ferrous Fe. The faint pink color should persist for at least 5 min. Add 10 cc of KCNS or $(NH_4)CNS$ soln, dilute with $H_2O$ to 100 cc and mix thoroly. Compare the color immediately with a series of standards, prepared side by side with the sample, in similar tubes. Prepare the standards from the standard ferric sulfate soln [Reagent **2(v)**] so as to have a range of from 0.000005 g Fe to 0.00004 g Fe (0.5–4.0 cc. Transfer the desired volumes of the standard ferric sulfate soln to 100-cc Nessler tubes containing 50 cc each of an acid soln (made up by dissolving 8 g of $(NH_4)_2SO_4$ in $H_2O$, adding 20 cc of $H_2SO_4$, cooling, diluting with $H_2O$ to 200 cc, and mixing). Add a drop of 0.1 *N* $KMnO_4$ soln (or sufficient to yield a pink color that will persist for 5 min.), and then 10 cc of the thiocyanate soln. Finally dilute all standards with $H_2O$ to 100 cc and mix each thoroly.

NOTE.—For a single sample it is more convenient to run the standard Fe soln from a buret into a Nessler tube, containing 50 cc of acid soln (made by dissolving 8 g of $(NH_4)_2SO_4$ in $H_2O$, adding 20 cc of $H_2SO_4$, cooling and diluting with $H_2O$ to 200 cc, and mixing), a drop of 0.1 *N* $KMnO_4$ soln, 10 cc of the thiocyanate soln, and then dilute with distilled $H_2O$ until the depth of the color produced after diluting 100 cc and mixing, exactly matches that of the sample. From the buret reading calculate the amount of Fe. When using standards, the color comparisons must be made immediately.

Calculate the total Fe found to $Fe_2O_3$ and report as such. Calculate the $TiO_2$ equivalent by multiplying the $Fe_2O_3$ result by the factor 1.003 and subtract this figure from the total $TiO_2$ as determined in **19** and report the remainder as $TiO_2$.

Report all results on the dry or moisture-free basis.

## MIXED OR COMPOSITE PIGMENTS

**21**

### MOISTURE[1] (MATTER VOLATILE AT 105–110°)

Place 1–2 g of the sample in a wide-mouthed, short weighing tube provided with a glass stopper. Heat with the stopper removed for 2 hours at a temp. between 105 and 110.° Insert the stopper, cool, and weigh. Calculate the loss in weight as moisture (matter volatile at 105–110°).

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[1] On an extracted and dried pigment, this determination is of little value. If the original paint contained gypsum, a part of the combined $H_2O$ of the latter will be driven off in the drying of the extracted pigment and in the "moisture" determination.

### 22 LOSS ON IGNITION

Ignite 1 g of the pigment in a porcelain crucible over a Meker burner to constant weight.[1]

### 23 INSOLUBLE MATTER

Moisten 1 g of the sample with a few drops of alcohol, cover, add 40 cc of HCl (1+1), boil gently 5–10 min. Wash off cover, evaporate to dryness, and heat at about 150° for ½–1 hour to dehydrate the residue. Moisten the residue with 4 cc of strong HCl, allow to stand a few min., dilute with 100 cc of hot $H_2O$, boil a few min., filter hot thru paper, wash with hot $H_2O$ (till washings give no test for Pb and Cl). Ignite the paper and residue in a Pt or porcelain crucible, cool, and weigh total insoluble matter.[2] (The insoluble matter may be filtered off on a Gooch crucible, washed with hot $H_2O$, dried at 105°, cooled, and weighed; then ignited, cooled, and weighed, when it is desired to get the loss on ignition (combined $H_2O$, organic matter, etc.) of same, or the insoluble matter is not to be further examined.) If the sample contains Ti pigment, practically all the $TiO_2$ will be found in the insoluble matter along with $BaSO_4$ and siliceous matter. The $TiO_2$ may be determined in the insoluble matter or in a separate portion of the original sample by the method described in **19** and **20**. To determine $BaSO_4$, mix the ignited insoluble matter with about 10 times its weight of anhydrous $Na_2CO_3$ (grinding the mixture in an agate mortar if necessary), and fuse the mixture in a covered Pt crucible, heating about 1 hour. Let cool, place crucible and cover in a 250 cc beaker, add about 100 cc of $H_2O$, and heat until the melt is disintegrated. Filter on paper (leaving crucible and cover in beaker) and wash the beaker and filter thoroly with hot $H_2O$ to remove soluble sulfates. Place the beaker containing the crucible and cover under the funnel, pierce the filter with a glass rod, and wash the residue into the beaker by means of a jet of hot $H_2O$. Wash the paper with hot dilute HCl (1+1) and then with hot $H_2O$. Remove crucible and cover, washing them with a jet of hot $H_2O$ and removing any adhering precipitate. Add cautiously 20 cc of concentrated $H_2SO_4$ and evaporate until fumes of $H_2SO_4$ are evolved and the precipitated matter is dissolved. *Cool*, add cautiously, with stirring, about 100 cc of $H_2O$, and boil a few minutes. Let the precipitate settle, filter on a weighed Gooch crucible, wash with hot $H_2O$, ignite, cool, and weigh as $BaSO_4$. Subtract the sum of the percentages of $BaSO_4$ and $TiO_2$ from the percentage of total insoluble matter and report the result as the percentage of insoluble siliceous matter.[3]

If it is desired to examine the siliceous matter, unite the filtrates from the $Na_2CO_3$ fusion and the $BaSO_4$ precipitate, acidify with HCl, evaporate to dryness, and proceed as in a silicate analysis, taking cognizance of any $TiO_2$ that may be found, if Ti pigment were originally present.

### 24 TOTAL LEAD ANTIMONY

Unite the filtrate and washings (total volume 150–200 cc) from the total insoluble matter, pass $H_2S$ into the soln until it is saturated, add an equal volume of $H_2O$, and again saturate with $H_2S$. Filter, wash with $H_2O$ containing a little $H_2S$, dissolve in hot $HNO_3$ (1+3), washing the paper with hot $H_2O$; add 10–20 cc of $H_2SO_4$ (1+1), evaporate until copious fumes of $H_2SO_4$ are evolved; cool, add about 75 cc of $H_2O$, and then about 75 cc of 95% ethyl alcohol. Stir, let settle, filter on a Gooch crucible, wash with dilute alcohol, and dry in an oven at 105–110°; or, ignite gently in a radiator[1] or muffle, cool, and weigh as $PbSO_4$. Calculate to PbO.[2]

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[1] This determination may serve as a rough or approximate check in many cases on the $CO_2$, $H_2O$, etc.

[2] See Ref. 3 under 12.

[3] Any soluble $Al_2O_3$ ($Fe_2O_3$) and in most cases MgO, and sometimes some CaO, come from the siliceous pigment used. MgO generally denoted the presence of asbestine.

If the pigment contains Sb, filter and wash the sulfide precipitate as above; then wash the precipitate with a fine jet of $H_2O$ from the paper into a porcelain dish or casserole, add 25 cc of $NH_4$-polysulfide [Reagent **2**(i)], cover the vessel, and warm the mixture at 40–60° for 10–15 min. with frequent stirring. Wash off cover, filter thru the paper used in the first case, and wash with 2–3% $Na_2S$ or $(NH_4)_2S$ soln. Discard the filtrate. Dissolve the residue in hot dilute $HNO_3$ (1+3), and determine the lead as $PbSO_4$, as described above. Or, the original sulfide precipitate may be discarded and the Pb determined on a separate portion of the pigment as follows: To 1 g of the sample in a covered beaker, add 40 cc of HCl (1+1) and boil gently for 5–10 min. Wash off cover and evaporate to dryness. Moisten the residue with a few drops of HCl, add about 50 cc of hot $H_2O$, boil a few minutes, filter hot thru paper, and wash with hot $H_2O$ until washings give no test for Pb. (If the sample contains no insoluble matter, the filtration is omitted.) To the filtrate add 20 cc of $H_2SO_4$ (sp. gr. 1.84) and evaporate until dense white fumes of $H_2SO_4$ are copiously evolved. Allow to cool, but not below 60°, and then add slowly 50 cc of $H_2O$ while the soln is agitated. Heat to boiling for several min. in order to insure complete soln of Sb-sulfate. Allow the $PbSO_4$ to settle out until the supernatant liquid is clear, not letting the temp. fall below 60°. If the liquid does not clear quickly it must be heated longer. When clear, pour the soln thru a weighed porcelain Gooch crucible with asbestos mat, decanting the soln as completely as possible without allowing more than a very small amount of $PbSO_4$ to go over into the crucible. Now add 10 cc more of $H_2SO_4$ (sp. gr. 1.84) to the $PbSO_4$ in the original beaker, and boil for several min. Cool, add slowly 30 cc of $H_2O$, and again heat to boiling for a few minutes; allow the soln to cool to about 60° and completely transfer the $PbSO_4$ to the Gooch crucible. Wash with "lead acid" [Reagent **2**(j)] to remove soluble sulfates and finally wash free of acid with dilute alcohol (equal parts of ethyl alcohol or denatured alcohol and $H_2O$). Dry in an oven at 105–110°, or ignite gently in a radiator or muffle. Calculate to PbO, or determine as chromate as described below.

If soluble compounds of Ba or Ca are present, $BaSO_4$ and $CaSO_4$ will be included with the $PbSO_4$. If soluble $SiO_2$ is present, it will also be included with the $PbSO_4$. In such cases, the $PbSO_4$ precipitate after washing with dilute alcohol may be dissolved in acid $NH_4$ acetate [Reagents **2**(**h**)] and the Pb determined as $PbCrO_4$, as described below. For ordinary work, the amount of $BaSO_4$ dissolved by the acetate treatment may be disregarded.

If the pigment contains no soluble Sb, Ba, or Ca compounds, the Pb may be determined directly on the original pigment, as follows: To 1 g of the sample in a covered beaker, add 25 cc of $HNO_3$ (1+1), and boil gently a few min. Wash off cover, evaporate to dryness on a steam bath, moisten with $HNO_3$, add hot $H_2O$, and heat a few min. Filter, and wash with hot $H_2O$ until washings are Pb-free. Add 10–20 cc of $H_2SO_4$ (1+1) to the clear soln, evaporate, and determine Pb as $PbSO_4$, as above described.

In the absence of soluble compounds of Sb, Fe, Al, and Ba, the following procedure may be used: Treat 1 g of the original pigment with 25 cc of $HNO_3$ (1+1) and proceed as above. To the clear soln, diluted to 200 cc add $NH_4OH$ in slight excess, acidify with acetic acid, and add 4–6 cc more of this acid; heat to boiling and add 10–15 cc of a 10% soln of $K_2Cr_2O_7$. Heat until the yellow precipitate assumes an orange color and let settle. Filter on a weighed Gooch crucible and wash by decantation until the washings are colorless, finally transferring all the precipitate. Wash with 95% alcohol and then with ether; dry to constant weight at 110°, cool, and weigh $PbCrO_4$. Calculate to PbO.

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[1] *U. S. Geological Survey Bulletin* **700**, p. 33 (1919).

[2] It is not possible to determine the amount of basic lead carbonate and lead sulfate when carbonates or soluble sulfates of other metals, such as calcium, are present. Also neither basic lead barbonate nor basic lead sulfate is a definite compound.

## 25 ANTIMONY OXIDE

### *Method I*

(In the ous condition)

Transfer 0.3 g of a straight antimony oxide pigment, or 0.5 g of a mixed pigment, to a 500 cc Pyrex Erlenmeyer flask, and add 15 cc of $H_2O$ and 25 cc of HCl. Cover with a watch-glass, warm on the steam bath 10–15 min. to dissolve the antimony oxide, wash off cover, and add 250 cc of $H_2O$ and 15 cc of $H_2SO_4$. Boil 2 min., cool to 10–15°, and titrate to a faint pink tint with 0.1 *N* $KMnO_4$ soln [Reagent **2(k)**]. Calculate to $Sb_2O_3$.

### 26 *Method II*

(In ous and ic condition)

Transfer 0.3 g of a straight $Sb_2O_3$ pigment, or 0.5 g of a mixed pigment, to a 500 cc Pyrex Erlenmeyer flask, add 15 cc of $H_2SO_4$ (sp. gr. 1.84), 10 g of $K_2SO_4$, and a 9 cm filter paper (to furnish C to act as a reducing agent). Place a funnel in the neck of the flask, and heat until the soln becomes colorless. Cool, wash off the funnel, dilute to 250 cc with $H_2O$, add 20 cc of HCl, and boil 2 min.; cool to 10–15°, and titrate to a faint pink tint with 0.1 *N* $KMnO_4$ soln.[1]

### 27 *Method III*

(For use in the presence of appreciable amounts of iron)

Treat 1 g of the mixed pigment, or 0.3 g of a straight $Sb_2O_3$ pigment, in a covered 250 cc beaker with 5 cc of $H_2O$ and 20 cc of HCl (sp. gr. 1.19); heat on the steam bath for 15 min., cool, wash off cover, add 3 g of tartaric acid and 100 cc of hot $H_2O$, and digest a few minutes. Filter, catching the filtrate in a 500 cc Pyrex Erlenmeyer flask; wash thoroly with hot $H_2O$, dilute to 300 cc with hot $H_2O$, and pass in $H_2S$ until the precipitation is complete. (If the sample contains no insoluble matter, dissolve directly in a 500 cc Pyrex Erlenmeyer flask, add tartaric acid, dilute, and pass in $H_2S$.) Filter, wash with $H_2O$ containing $H_2S$ until free from HCl, return paper and precipitate to the Erlenmeyer flask, add 15 cc of $H_2SO_4$ (sp. gr. 1.84) and 10 g of $K_2SO_4$, place a funnel in the neck of the flask, and heat until the soln is colorless. Cool, wash off the funnel, dilute to about 250 cc with $H_2O$, add 20 cc of HCl (sp. gr. 1.19), boil for 2 or 3 min., cool to about 10°, and titrate to a faint pink tint with 0.1 *N* $KMnO_4$ soln [Reagent **2(k)**]. Calculate the total antimony to $Sb_2O_3$.[2]

## 28 SOLUBLE BARIUM

Boil the combined filtrate and washings, reduced in volume by evaporation if need be, from the PbS precipitate (Total Pb) to expel $H_2S$. Add a slight excess of $H_2SO_4$ (1+4) over the amount required to precipitate the Ba, heat to boiling, let stand on a steam bath about 1 hour, filter on a weighed Gooch crucible, wash with hot $H_2O$, dry, ignite, cool, and weigh $BaSO_4$.[3] Calculate to BaO.

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[1] If the digestion with $H_2SO_4$ and $K_2SO_4$ (plus filter paper) is continued after the solution becomes colorless, some of the antimony may be oxidized from the *ous* to the *ic* condition. In such cases, cool, wash off the funnel, dilute to 100 cc with $H_2O$, add 1–2 g of $Na_2SO_3$, and boil until all the $SO_2$ is expelled. This is shown when no blue color is obtained with starch-iodate paper [ Reagent **2(r)**]; the volume will be reduced about one-half. Dilute to 250 cc with $H_2O$, add 20 cc of HCl (sp. gr. 1.19), and boil 2 min.; cool to 10–15°, and titrate to a faint pink tint with 0.1 *N* $KMnO_4$ soln. Calculate total Sb to $Sb_2O_3$. Subtract the $Sb_2O_3$ found by the procedure given in the first paragraph under **25** from the total $Sb_2O_3$ and calculate the residual $Sb_2O_3$ to $Sb_2O_5$.

[2] See Ref. 1 above, omitting the last two sentences.

[3] This will include any $BaSO_4$ that may have been dissolved as such. The weighed precipitate should be tested for $CaSO_4$, and if present, it should be removed by treating with hot dilute HCl, filtering, washing, igniting, and again weighing. Also see Ref. 3 under **12**.

### 29 ALUMINA ($Fe_2O_3$ $TiO_2$, $P_2O_5$)

Boil the filtrate from the PbS to expel $H_2S$, add a few drops of $HNO_3$, and continue the boiling a few min. to oxidize any Fe that may be present. In case soluble Ba was present, use the filtrate from that determination. To the soln containing at least 5 g of $NH_4Cl$ per 200 cc of soln, or an equivalent amount of HCl, add a few drops of methyl red (0.2% alcoholic soln) and heat just to boiling. Carefully add dilute $NH_4OH$ dropwise until the color of the soln changes to a distinct yellow. Boil the soln for 1 to 2 min. and filter at once. Wash the precipitate thoroly with hot 2% $NH_4Cl$ soln.[1] Ignite the precipitate, cool, and weigh as $Al_2O_3$.[2]

## TOTAL ZINC

### 30 *Method I*

To the combined filtrate and washings from the alumina precipitate, add sufficient $NH_4Cl$ to give 5 g per 100 cc of soln, and then add 1 g of $NH_4$-acetate.[3]

Render slightly acid with acetic acid and pass in a current of $H_2S$ to saturation. Allow the precipitate to settle completely, filter on paper, and wash with a 2% soln of acetic acid saturated with $H_2S$. Transfer the precipitate and filter to the vessel in which the precipitation was effected, add 30 cc of $H_2O$ and 10 cc of HCl, heat until all Zn is in soln, add 200 cc of $H_2O$ and a small piece of litmus paper; add $NH_4OH$ until slightly alkaline, render just acid with HCl, then add 3 cc of HCl, heat nearly to boiling, and titrate with standard $K_4Fe(CN)_6$ soln as in standardizing that soln [Reagent **2**(1)].

### 31 *Method II*

Zinc may be determined directly on the original sample as follows:[4] Weigh accurately about 1 g (or an amount that will give a buret reading approximately equal to that obtained in the standardization) of the pigment, transfer to a 400 cc beaker, add 30 cc of HCl (1+2), boil a few min., add 200 cc of $H_2O$ and a small piece of litmus paper; add $NH_4OH$ until slightly alkaline, render just acid with HCl, then add 3 cc of HCl, heat nearly to boiling, and titrate with standard $K_4Fe(CN)_6$ soln as in standardizing that soln [Reagent **2**(1)].

### 32 *Method III*

When Fe is present, total Zn may be determined directly on the original sample as follows:[5] Weigh accurately about 1 g (or an amount that will give a buret reading approximately equal to that obtained in the standardization) of the pigment, transfer to a 250 cc beaker, moisten with alcohol, add 30 cc of HCl (1+2), boil for 2 or 3 min., and add about 100 cc of $H_2O$. Add about 2 g of $NH_4Cl$, make slightly alkaline with $NH_4OH$, heat to boiling, let settle on steam bath, filter into a 400 cc beaker, and wash the residue once with hot $H_2O$. Remove the 400 cc beaker, pour dilute HCl on the residue, catching the filtrate therefrom in the 250 cc beaker, and wash a few times with hot $H_2O$. Add to this filtrate 1 g of $NH_4Cl$ and make slightly alkaline with $NH_4OH$, boil, let settle, filter on paper used for first filtration, and wash thoroly with hot $H_2O$, catching the filtrate and washings in the 400 cc beaker containing the first filtrate. Add a small piece of litmus paper, acidify with HCl, add 3 cc of HCl, heat nearly to boiling, and titrate with standard $K_4Fe(CN)_6$ as above.

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[1] For very accurate work, or when the precipitate is large, the precipitate should be dissolved in HCl (1+1) and the precipitation repeated.

[2] This precipitate may also contain $Fe_2O_3$, $TiO_2$, and $P_2O_5$.

[3] Gooch, Representative Procedures in Quantitative Chemical Analysis, 1st ed., p. 107.

[4] If the sample contains Sb, it should be precipitated by $H_2S$ in the hot acid soln, filtered off, washed, and the filtrate neutralized, etc., for Zn. The $H_2S$ precipitate may also contain PbS. If no sulfide separation is made, any Cd present will be counted as Zn.

[5] See Ref. 5 under **23**.

### 33 *Method IV*

With pigments containing ZnO and ZnS, the ZnO may be determined as follows: Weigh accurately 2.5 g of the pigment, transfer to a 250 cc graduated flask, moisten with a few drops of alcohol, add about 200 cc of 2-3% acetic acid, shake vigorously, and let stand for 30 min. at room temp., shaking once every 5 min. Then let stand at room temp. at least 5 hours, preferably overnight. Fill to the mark with 2–3% acetic acid, mix, filter thru a dry paper, discard the first 25 cc and transfer 100 cc of the filtrate (corresponding to 1 g) to a 400 cc beaker. To the clear soln add 30 cc of HCl (1+2), 100 cc of $H_2O$, and a small piece of litmus paper; add $NH_4OH$ until slightly alkaline, render just acid with HCl, then add 3 cc of HCl, heat nearly to boiling, and titrate with $K_4Fe(CN)_6$ as above. Calculate the percentage of ZnO (any $ZnCO_3$ or $ZnSO_4$ is included in the ZnO). Subtract this result from the percentage of total Zn as ZnO, and calculate the difference to ZnS.

### 34 SOLUBLE CALCIUM

Heat the united filtrate and washings, reduced in volume if need be, from the ZnS precipitate, to boiling, and add 1 cc of $NH_4OH$ and an excess of a hot saturated $NH_4$-oxalate soln. Continue the boiling until the precipitate becomes granular; let stand about 1 hour, filter, and wash with hot $H_2O$. Ignite, cool, and weigh as CaO;[1,2] or place the beaker in which the precipitation was made under the funnel, pierce the apex of the filter with a stirring rod, and wash the precipitate into the beaker with hot $H_2O$, pouring warm $H_2SO_4$ (1+4) thru the paper and washing a few times. Add about 30 cc of $H_2SO_4$ (1+4), dilute to about 250 cc, heat to 90°, and titrate at once with standard (0.1 $N$) $KMnO_4$ soln (the temp. of the soln should not be below 60° when the end point is reached. See Reagents). Calculate to CaO.[3] (The Fe value of $KMnO_4 \times 0.502 =$ CaO value.)

### 35 SOLUBLE MAGNESIUM

Acidify the filtrate from the Ca precipitate with HCl, add 10 cc of a saturated soln of $Na(NH_4)HPO_4$ and $NH_4OH$ dropwise, with constant stirring. When the crystalline $(NH_4)MgPO_4$ has formed, add 5 cc excess of $NH_4OH$. Allow the soln to stand in a cool place for not less than 4 hours, preferably overnight;[4] filter and wash with $H_2O$ containing 2.5% of $NH_3$. Dissolve the precipitate in a small quantity of hot dilute HCl, dilute the soln to about 100 cc with $H_2O$, add 1 cc of a saturated soln of $Na(NH_4)HPO_4$ and $NH_4OH$ dropwise, with constant stirring, until the precipitate is again formed as described, and then add 5 cc excess of $NH_4OH$. Let the precipitate stand in a cool place for not less than 2 hours, filter on a Gooch crucible, wash with $H_2O$ containing 2.5% of $NH_3$, ignite, cool, and weigh as $Mg_2P_2O_7$.[5] Calculate to MgO.

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[1] Care must be exercised in this washing, as 1000 cc of boiling water will dissolve over 0.01 g of $CaC_2O_4$.

[2] For more accurate work, the $CaC_2O_4$ precipitate should be ignited, cooled, cautiously moistened with $H_2O$, redissolved in HCl and the soln diluted to 100 cc. Then $NH_4OH$ should be added in slight excess, the liquid boiled, and filtered and washed if a precipitate appears. Then reprecipitate the Ca with $NH_4OH$ and $(NH_4)_2C_2O_4$ as above, filter, wash, ignite, cool, and weigh; or, titrate as described. See also Ref. 3 under **12.**

[3] See Ref. 3 under **12,** also Refs. 2 and 3 under **26.**

[4] The less the amount of Mg present, the longer the precipitate must be allowed to settle.

[5] If the sample contained Mn, it will be caught in large part with the $Mg_2P_2O_7$. If desired, Mn may be determined by dissolving the $Mg_2P_2O_7$ in $HNO_3$ and applying the bismuthate method.

### 36 CARBON DIOXIDE

Use from 1 to 2 g of the pigment, depending upon the probable $CO_2$ content,[1] following either of the methods described under the Determination of Carbon Dioxide in the Standard Methods of Chemical Analysis of Limestone, Quicklime and Hydrated Lime (A.S.T.M. Designation: C 25) of the American Society for Testing Materials.[2]

### 37 TOTAL SOLUBLE SULFUR COMPOUNDS[3]

Treat 1 g of the pigment in a 400 cc beaker with 10 cc of $H_2O$, 10 cc of HCl saturated with Br, and 5 g of $NH_4Cl$, digest (covered) on a steam bath for 5 min., dilute with hot $H_2O$ to about 200 cc, boil for 5 min., filter to separate any insoluble matter, and wash thoroly with hot $H_2O$. Nearly neutralize the clear soln in a covered beaker with NaOH soln, complete the neutralization with dry $Na_2CO_3$, and add about 2 g more of this reagent. Boil 10–15 min., wash off cover, let settle, filter, and wash with hot $H_2O$. Re-dissolve the precipitate in HCl (1+1), reprecipitate with $Na_2CO_3$ as above, filter, and wash thoroly with hot $H_2O$. Acidify the united filtrates with HCl, adding about 1 cc in excess. Boil to expel Br, and to the clear boiling soln add slowly with stirring an excess of a 10% $BaCl_2$ soln. Let stand on a steam bath for at least 1 hour, filter on a weighed Gooch crucible, wash thoroly with boiling $H_2O$, dry, ignite at a dull red heat, cool, and weigh as $BaSO_4$. This will include soluble sulfates, $SO_3$ formed from $SO_2$ and the $SO_3$ that is formed from sulfide S.[4]

### 38 SOLUBLE SULFATE[5]

Treat 1 g of the pigment with 10 cc of $H_2O$, 10 cc of HCl, and 5 g of $NH_4Cl$. Boil until $H_2S$ is expelled, adding more HCl (1+1) if necessary; dilute with hot $H_2O$ to about 200 cc, boil for 5 min., filter to separate any insoluble matter, and wash thoroly with hot $H_2O$. Nearly neutralize the clear soln with NaOH soln and make a double precipitation with $Na_2CO_3$, as in preceding method, finally weighing as $BaSO_4$, as described above.[4]

### 39 SULFIDE SULFUR[6]

Place 0.5-1 g of the pigment in a flask with about 10 g of "feathered" or mossy Zn, and add 50 cc of $H_2O$; insert a stopper carrying a separatory funnel and an exit tube. Run in 50 cc of HCl from the funnel, having previously connected the exit tube to 2 absorption flasks in series; the first flask contains 100 cc of alkaline $Pb(NO_3)_2$ soln [Reagent **2(n)**], the second flask, 50 cc of the same soln as a safety device. After all the acid has run into the evolution flask, heat slowly, finally boiling until the first appearance of steam in the first absorption flask. Disconnect, let the PbS settle, filter, and wash with cold $H_2O$, then with hot $H_2O$ till neutral to litmus paper and washings give no test for Pb. Dissolve the PbS precipitate in hot, dilute $HNO_3$, and determine the Pb as $PbSO_4$. Calculate to S. For very rapid work, the evolved $H_2S$ may be absorbed in an ammoniacal $CdCl_2$ or $ZnSO_4$ soln [Reagent **2(o)**] contained in 2 flasks connected in series, the contents of the absorption flasks washed into a vessel with cold $H_2O$ and diluted to about 1 liter, acidified with HCl, and titrated with standard $KIO_3$ soln [Reagent **2**(**p**)], using starch indicator [Reagent **2**(**q**)].

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[1] If the sample is high in sulfide, *e.g.*, contains a high percentage of lithopone, grind 1–2 g of the pigment with dry $K_2Cr_2O_7$, transfer to the evolution flask, add 50 cc of $H_2O$, and run in $H_2SO_4$ (1+1) from the separatory funnel. Or, place at the front of the purifying and drying train a tube containing acidified $CuSO_4$ soln, $KMnO_4$ soln, or $CrO_3$ soln.

[2] 1929 Supplement to Book of A. S. T. M. Standards.

[3] See Ref. 3 under **12**.

[4] See Ref. 1 under **23**.

[5] See Ref. 3 under **12**.

[6] Evolution Method of W. G. Scott, White Paints and Painting Materials, p. 257; see also Blair, The Chemical Analysis of Iron. The percentage of sulfide sulfur can be calculated from the percentages of total Zn and Zn soluble in 2–3% acetic acid, assuming the sulfide to be ZnS. See **33**.

### 40 SULFUR DIOXIDE[1]

Transfer 10 g of the pigment to a suitable flask, insert a stopper fitted with a separatory funnel and a spray trap delivery tube,[2] and attach the latter to a condenser. Place about 150 cc of HCl (1 +3) in the funnel, the stopcock being closed,[3] and connect the other end of the condenser with a delivery tube which passes thru a 2-hole stopper and extends nearly to the bottom of an absorption flask; thru the other hole of the stopper connect a tube or flask to serve as a safety device. Place 25 cc of 0.05 *N* I soln [Reagent **2**(**s**)] in the absorption flask (dilute with $H_2O$ if need be) and 20 cc of 10% KI soln in the safety tube; fit stopper in the absorption flask. Open the stopcock and allow the acid to slowly enter the flask. Before all the acid is admitted, air (washed with NaOH soln) is forced thru the top of the separatory funnel (about 2 bubbles per second in the KI soln). Boil the soln 3 min. with the air passing thru, then remove the source of heat and pass air thru for 30 min. Disconnect the absorption vessels, wash the KI soln into the I soln, and titrate at once with 0.05 *N* $Na_2S_2O_3$ soln, using starch indicator. Run a blank determination in exactly the same manner except for the omission of the pigment. Subtract this figure from the previous one and calculate the final result to $SO_2$ (1 cc of 0.05 *N* I = 0.0016 g of $SO_2$).

### 41 MATTER SOLUBLE IN WATER

Transfer 2.5 g of the pigment to a graduated 250 cc flask, add 100 cc of $H_2O$ and boil for 5 min. Cool to room temp., dilute to the mark with $H_2O$, mix, and allow to settle. Filter the supernatant liquid thru a dry filter paper and discard the first 20 cc of the filtrate. Transfer 100 cc of the clear filtrate to a weighed dish, evaporate to dryness on a steam bath, dry for 1 hour in an oven at 105–110°, cool, and weigh. Calculate the percentage of water-soluble matter. (The nature of this may be determined by further examination, as the percentages of $SO_3$ and CaO may be indicative.)

## 42 CALCULATIONS

The calculation of the component pigments of a mixed or combination pigment may be difficult. Depending upon the complexity of the mixed pigment, certain assumptions must be made as to the composition or formulas of component pigments and as to the manner in which the acidic and basic radicles are combined. Add any $Al_2O_3$ ($Fe_2O_3$) found in the soluble portion to the siliceous matter and report the sum as "Insoluble siliceous matter," unless the soluble Al is high; in this case, an aluminate is probably present, and the $Al_2O_3$ should be reported as $Al_2O_3$. If a small amount of soluble Mg is found, it should also be added to the siliceous matter. If the soluble Mg is high, the presence of $MgCO_3$ is indicated, and the MgO is calculated to $MgCO_3$ as pointed out below. The insoluble siliceous matter reported should be based on the weight obtained on drying the total insoluble matter at 105° if the combined $H_2O$ contained therein is to be considered.

In the absence of ZnS or $TiO_2$, report $BaSO_4$ as $BaSO_4$. If ZnS is present, calculate the $BaSO_4$ equivalent by multiplying by 2.85; report sum of $ZnS + BaSO_4$ as "lithopone." If $TiO_2$ is present, calculate the $BaSO_4$ equivalent by multiplying by 3.17; report sum of $TiO_2+BaSO_4$ as "titanium pigment." Report residual $BaSO_4$ as $BaSO_4$. If $TiO_2$ is present and $BaSO_4$ is absent or is present in a smaller amount than would be indicated by the above factor, then report $TiO_2$ as $TiO_2$, and $BaSO_4$ as $BaSO_4$. If $CaCO_3$, $CaSO_4$, $BaCO_3$, and $MgCO_3$ are absent, calculate $CO_2$ to basic carbonate white lead, $(PbCO_3)_2.Pb(OH)_2$, and soluble $SO_3$ to $PbSO_4$. Any excess of Pb is calculated to PbO, added to the $PbSO_4$, and the sum is reported as basic $PbSO_4$; or, multiply the sum of $PbSO_4+PbO$ by 0.058 to obtain the ZnO; add this result to the $PbSO_4+PbO$ and report as basic sulfate white lead. (The ZnO factor is based on the assumption that the average composition of commercial basic sulfate white lead is: 78.5% $PbSO_4$, 16.0% PbO, and 5.5% ZnO.) Lead oxide (PbO) should not be reported except in the presence of $PbSO_4$, unless the entire analysis is reported in the elementary or oxide form.

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[1] This method is not applicable in the presence of sulfides decomposable under the conditions given.

[2] A Knorr $CO_2$ apparatus is convenient. In this case, the vertical condenser may be connected with an absorption tower containing the I soln, followed by the KI soln in a suitable tube.

[3] To minimize, if not eliminate, any possible oxidation by the air, add about 1 g (in one piece) of $NaHOC_3$ to the evolution flask, then add the acid directly to the flask, omitting the separatory funnel and the current of air. Boil the soln until about 50 cc of distillate has passed over.

If the sample contains $CO_2$ but no soluble $SO_3$, calculate total Pb to basic carbonate white lead, $(PbCO_3)_2.Pb(OH)_2$; calculate residual $CO_2$ to $CaCO_3$, then to $BaCO_3$ and $MgCO_3$ if soluble Ba and Mg should be present in sufficient amounts to indicate the presence of these carbonates. The $CO_2$ result will be an index of this. A small amount of residual CaO is probably from the siliceous matter and should be added to the insoluble siliceous matter.

A small amount of soluble Ba may be from the $CaCO_3$ used or may be due to the solubility of $BaSO_4$ if this compound is present in the original pigment. This Ba may be calculated to $BaSO_4$ and added to the $BaSO_4$ found in the insoluble matter.

If the sample contains soluble $SO_3$ but no $CO_2$, calculate CaO to $CaSO_4$ or $CaSO_4.2H_2O$; residual $SO_3$ to $PbSO_4$; add residual PbO to $PbSO_4$ and report sum as basic $PbSO_4$; or, multiply $PbSO_4+PbO$ by 0.058 and add the result to the $PbSO_4+PbO$, and report the total as basic sulfate white lead.

If the sample contains $CaCO_3$ ($MgCO_3$, $BaCO_3$) and also basic sulfate white lead, or $CaSO_4$ and basic carbonate white lead, or a mixture of these, it is not possible to determine or calculate the amount of $PbCO_3$ or $PbSO_4$ with any degree of certainty.[1] The presence of appreciable amounts of CaO and $SO_3$ in the water-soluble matter indicates the probable presence of $CaSO_4$ in the original pigment. The following arbitrary calculations may be made: calculate water-soluble $SO_3$ to $CaSO_4$ or $CaSO_4.2H_2O$, subtract this $SO_3$ from total soluble $SO_3$ and calculate the remainder to $PbSO_4$; calculate residual CaO to $CaCO_3$, and then residual $CO_2$ to $(PbCO_3)_2.Pb(OH)_2$. If there is an excess of $CO_2$, calculate to $MgCO_3$ or $BaCO_3$, if the amounts of soluble Mg and Ba indicate the probable presence of these carbonates. Add residual PbO to $PbSO_4$ and calculate, as above, to basic sulfate white lead. The procedure followed by the Federal Specifications Board should be noted.[2]

Report total Sb as $Sb_2O_3$.

Calculate sulfide S to ZnS, subtract the Zn equivalent to the S from the total Zn, then subtract the Zn required for the basic sulfate white lead, and report the remainder as ZnO.

Report moisture, loss on ignition, $SO_2$, and matter soluble in $H_2O$ directly.

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[1] See Ref. 2 under **16** and Ref. 3 under **12.**

[2] Federal Specifications Board Specification No. 10*b* for "White Paint and Tinted Paints Made on a White Base, Semipaste and Ready Mixed"; U. S. Bur. Standards *Circ.* **C89**, 3d ed., p. 2: "The total lead dissolved by dilute acetic acid and hot acid, ammonium acetate, weighed as lead sulfate, and this weight multiplied by the factor 0.883 shall be considered white lead. (It is not possible to determine the amount of $PbCO_3$ and $PbSO_4$ when carbonates or sulfates of other metals, such as Ca, are present. Also neither basic $PbCO_3$ nor basic $PbSO_4$ are definite compounds. The factor to convert $PbSO_4$ to $(PbCO_3)_2Pb(OH)_2$ is 0.854, to convert $PBSO_4$ to $PbSO_4\cdot PbO$ is 0.868, and to convert $PbSO_4$ to $(PbSO_4)_2\cdot PbO$ is 0.913. The arbitrary factor used under this specification is the mean of the largest and smallest of these 3 factors.)"

# X. LEATHERS—TENTATIVE

(These methods are essentially the same as the official and provisional methods of the American Leather Chemists Association.)

## VEGETABLE TANNED LEATHER

### 1 PREPARATION OF SAMPLE[1]

Reduce the leather by slicing, planing, cutting, shredding, or rasping to as fine a state of subdivision as is practicable. Avoid heating the sample during its preparation. Do not use the ordinary type of grinding mill. Spread out the prepared sample and allow it to return to atmospheric moisture condition; mix thoroly; and place in tightly covered containers.

### MOISTURE[2]

### 2 *Method I*

Place 5-10 g of the sample, as prepared under **1**, in a tared, wide, shallow weighing bottle (or a similar dish, which can be covered tightly), and dry in an electric oven for 15 hours at 100–102°. Cover the weighing bottle, cool in a desiccator containing concentrated $H_2SO_4$, and weigh. The moisture in the leather as received may be determined by quickly cutting a representative portion of the sample into small pieces and drying as directed without further preparation.

### *Method II—By Toluene Distillation*

### 3 APPARATUS

(**a**) 500 cc flask.—Erlenmeyer or distilling flask of Pyrex or other resistant glass.

(**b**) *Receiving tube.*—Graduated in tenths of a cc.

(**c**) *Liebig condenser.*—Sealed-in, straight-tube, about 25 cm (10 inches) long, with delivery tube approximately 9.5 mm (0.375 inch) in diameter.

Assemble the apparatus as shown in **XXVII, 3.** Before each distillation clean the condenser and receiving tube with $CrO_3$-$H_2SO_4$ mixture; rinse thoroly with $H_2O$, then with alcohol; and dry in an oven or with a current of air. Calibrate the receiving tube by distilling toluene containing known quantities of $H_2O$. Read the volume of $H_2O$ to 0.01 cc.

### 4 DETERMINATION

Weigh 20 g of the prepared sample and transfer to the distilling flask. Immediately add about 200 cc of dry toluene having a boiling point, under normal pressure, of 110–112°, and connect the flask with the receiving tube and condenser. Fill the receiving tube with toluene, pouring it thru the condenser. Heat the distilling flask gently and distil at the rate of about 4 drops per second for exactly 2 hours. At the end of 1, 1.25, 1.5, 1.75, and 2 hours' distillation, wash down the condenser by pouring toluene in at the top while brushing thoroly with a tight-haired, close fitting tube brush that has been boiled previously in toluene. (A long handle may be made by fastening to the brush a piece of heavy Cu wire.) At the end of 2 hours disconnect the receiving tube, dislodge any drops of $H_2O$ on the inside by rubbing with a piece of light Cu wire twisted at one end into a loop, and allow the tube to come to room temp. Read the volume of $H_2O$ to 0.01 cc and make such calibration correction as may be necessary. Assuming that 1 cc of $H_2O$ weighs 1 g, calculate the percentage of moisture.

## 5 TOTAL ASH[3]

Incinerate slowly 5 g of the sample, as prepared under **1**, at a dull red heat. If difficulty is experienced in burning off the C, leach the residue with hot $H_2O$, filter on an ashless filter, dry and ignite the filter and residue, add the filtrate, evaporate to dryness, and ignite. Cool in a desiccator containing concentrated $H_2SO_4$ and weigh.

The ash may be examined for acids and bases by any suitable method. Al, Mg,[4] Na, Ba, Ca, and Pb are the bases, and HCl and $H_2SO_4$ are the acids which it may be necessary to determine.

## 6 INSOLUBLE ASH[3]

Air-dry the leather remaining after the extraction of water-soluble material as directed under **9** and weigh. Incinerate slowly a portion equal to exactly $\frac{1}{3}$ of the total weight until all C is burned off, cool in a desiccator containing concentrated $H_2SO_4$, and weigh. Calculate the insoluble ash on the basis of the original leather represented.

## 7 PETROLEUM ETHER EXTRACT[5]

Place 5 g of the leather, as prepared under **1**, in a fat-free paper thimble, cover with a layer of fat-free cotton, and extract in a Johnson or Soxhlet extractor for 8–10 hours with petroleum ether, distilling between 50 and 80°. Heavily greased leathers (containing 15% or more fat) will require the maximum time. Remove the receiving flask, evaporate the petroleum ether on a steam bath, and dry the residue at 98–100° for periods of $\frac{1}{2}$ hour each until a practically constant weight is obtained. Avoid prolonged continuous heating, resulting possibly in the partial volatilization or oxidation of the extract.

## 8 MINERAL ACIDITY[6]

### (Modified Procter and Searle method[7])

Weigh 2 g of the leather, as prepared under **1**, into a Pt dish; add 40 cc of a 0.1 *N* $Na_2CO_3$ soln; mix thoroly; and evaporate to complete dryness on a steam bath. Incinerate the residue at a dull red heat, preferably in a muffle furnace, until the C has been nearly burned off; let cool; moisten carefully with hot $H_2O$, adding about 25 cc; and break up lumps with a glass rod. Filter into a 300 cc flask thru an ashless paper and wash 4 or 5 times with hot $H_2O$. Return the filter paper and residue to its dish, dry, and ignite until all C is burned off. Cool, and add to the residue from a buret a quantity of 0.1 *N* $H_2SO_4$ exactly equivalent to the $Na_2CO_3$ originally added. Cover the dish and place on a steam bath for 30 min. Filter, if necessary, into the flask containing the first filtrate, washing the paper thoroly with hot $H_2O$ until free from acid. Cool the soln and add 2 or 3 drops of methyl orange indicator. If the soln is alkaline no further titration is necessary, and the acidity is stated as "none." If the soln is acid, titrate to a distinct yellow color with the 0.1 *N* $Na_2CO_3$ soln. Express the result as percentage of $H_2SO_4$. With each set of determinations run a blank thru the entire procedure, using the standard solns. If the blank is over 0.3 cc, repeat the determinations.

## 9 EXTRACTION OF WATER-SOLUBLE MATERIAL[8]

Weigh 30 g of the leather, prepared as directed under **1**. (If the fat content of the sample, as determined by the petroleum ether extract, is more than 6%, extract the 30 g charge with petroleum ether, distilling between 50 and 80°, and allow the petroleum ether to evaporate spontaneously from the charge before proceeding with the extraction of water-soluble material.) To the charge add sufficient $H_2O$ to soak thoroly and cover it, and mix well. Transfer the leather and extract to a percolator that may be kept at 50°. (The Reed-Churchill extractor[9] is especially convenient.) Extract at 50° by percolating with $H_2O$ at 50°, collecting 2 liters of percolate in 3 hours. Cool to room temp., dilute to exactly 2 liters, and mix thoroly.

To the extract, prepared as above, add a few drops of toluene to prevent fermentation and reserve for the determination of glucose, soluble solids, and soluble non-tannins.

### GLUCOSE[10]

### 10 REAGENTS

(a) *Di-potassium phosphate.*—Use only $K_2HPO_4$ that is practically free from primary and tertiary salts, has been dried in thin layers at 98-100° for 16 hours, and kept in tightly stoppered bottles. A soln of the salt should have a pH value of about 9.0 and give a barely perceptible pink with phenolphthalein indicator.

(b) *Neutral lead acetate soln.*—Prepare as directed under **XXXIV, 18 (d).**

(c) *Soxhlet's modification of Fehlings soln.*—Prepare as directed under **XXXIV, 31.**

(d) *Phenolphthalein soln.*—Dissolve 0.5 g of phenolphthalein in 100 cc of 95% alcohol.

(e) *Tartaric acid.*—Grind pure tartaric acid to a fine powder.

### 11 PREPARATION OF SOLUTION

To 200 cc of the leather extract, prepared as directed under **9**, add by means of a pipet 25 cc of the neutral Pb acetate soln. Shake frequently for 5–10 min., then filter at once thru a dry, folded filter, returning the filtrate until it is clear. Keep the containers and funnel covered during these operations. Add to the filtrate 5.5 g of the dried $K_2HPO_4$. (The quantity of $K_2HPO_4$ must be not less than 4.5 nor more than 6.5 g.) Shake frequently for 3–5 min., until all the phosphate has dissolved; then filter thru a dry, folded filter, returning the first runnings until the filtrate clears, and letting the funnel drain well. Pipet 150 cc of the filtrate into a 500 or 600 cc Erlenmeyer flask, and add by means of a pipet 7.5 cc of HCl (sp. gr. 1.18). Also add about 25 mg of powdered stearic acid or 5–10 drops of kerosene to control frothing, and boil under a reflux condenser for exactly 2 hours. (Should the soln foam when it begins to boil, turn off the flame at the first indication of foaming, and when foaming subsides relight immediately. No further trouble should be experienced. After hydrolysis the acid soln may be allowed to stand at laboratory temp. overnight without risk of loss of sugar.) Cool to 10–15°, add 2 drops of phenolphthalein indicator, carefully neutralize with NaOH (1+1) added from a buret, and then add 0.5 cc in excess. *Without delay* transfer to a 200 cc volumetric flask, complete to volume with $H_2O$, and filter thru a double filter, returning the filtrate until it is clear. *During filtration keep the filtrate just acid by the addition from time to time of small quantities of pulverized pure tartaric acid. Immediately* determine the dextrose in the soln.

### 12 DETERMINATION

Pipet 50 cc of the soln, as prepared under **11**, into a mixture of 25 cc of the Cu soln and 25 cc of the alkaline tartrate soln and proceed as directed under **XXXIV, 38.** Express the results as percentage of glucose on the leather basis, the 50 cc aliquot being equivalent to 0.5 g of leather.

### 13 SOLUBLE SOLIDS

If the $H_2O$ extract, as prepared under **9**, is clear, proceed as directed under **XI, 2**; if cloudy, proceed as directed under **XI, 4.**

### 14 SOLUBLE NONTANNINS

Determine as directed under **XI, 7.**

### 15 SOLUBLE TANNIN

The percentage of soluble tannin is the difference between **13** and **14.**

### 16 NITROGEN

Determine as directed under **II, 20.**

### 17 HIDE SUBSTANCE

Multiply the percentage of N, as obtained under **16,** by the factor 5.62, to convert to the percentage of hide substance.

### 18 COMBINED TANNIN

Deduct the sum of the percentages of moisture, under **2** or **3**; insoluble ash, under **6**; petroleum ether extract, under **7**; soluble solids, under **13**; and hide substance, under **17,** from 100. The remainder is the percentage of combined tannin.

## SELECTED REFERENCES

[1] J. Am. Leather Chem. Assoc., **13,** 232 (1918); **14,** 321 (1919); **18,** 154 (1923); **23,** 412 (1928).

[2] Ibid., **16,** 547 (1921); **17,** 262 (1922); **19,** 568 (1924); **20,** 334 (1925); **21,** 435 (1926); **22,** 265 (1927); J. Assoc. Official Agr. Chem., **10,** 31, 143 (1927).

[3] J. Am. Leather Chem. Assoc., **13,** 7 (1918); **14,** 243 (1919); **15,** 130, 270 (1920).

[4] Ibid., **16,** 595 (1921); **17,** 274, 592 (1922).

[5] Ibid., **14,** 140, 499, 507 (1919); **16,** 458 (1921); **17,** 292, 540 (1922); J. Soc. Leather Trades Chem., **4,** 300 (1920).

[6] Procter, Leather Industries Laboratory Book, 2nd ed., 1908, p. 369; J. Am. Leather Chem. Assoc., **14,** 330 (1919).

[7] J. Am. Leather Chem. Assoc., **17,** 88 (1922); **18,** 430 (1923).

[8] Ibid., **11,** 219 (1916); **13,** 142 (1918); **14,** 488 (1919); **15,** 581 (1920); **16,** 124, 264, 491 (1921); **17,** 220 (1922).

[9] Ibid., **14,** 133 (1919).

[10] Ibid., **7,** 645 (1912); **9,** 421 (1914); **15,** 411 (1920); **16,** 480 (1921); **17,** 284 1922); **18,** 262, 459 (1923); **19,** 237, 339 (1924).

# XI. TANNING MATERIALS[1]—TENTATIVE

(These methods are essentially the same as the official and provisional methods of the American Leather Chemists Association.)

## EXTRACTS

**1**

### PREPARATION OF SOLUTION

(a) *Solid and powdered extracts.*—Grind the sample, if necessary, as rapidly as possible in a porcelain mortar until all will pass a 10-mesh sieve of Cu or brass; mix thoroly; and bottle. Weigh rapidly a quantity of sample containing 4 g of tannin (not less than 3.75 g nor more than 4.25 g).[2] Transfer to 100 cc of $H_2O$ at 85°, place on a steam bath, cover, and heat. Stir frequently until a homogeneous soln or suspension is obtained. Wash into a 1 liter flask with 800 cc of $H_2O$ at 85°. Allow to cool overnight at a temp. not below 19°; bring to 20° by placing the flask in $H_2O$, the temp. of which is not below 19°; and dilute to 1 liter.

(b) *Liquid extracts.*—Let the sample come to room temp., mix thoroly, and weigh rapidly a charge yielding the same quantity of tannin as specified under (a). Dissolve by washing into a liter flask with 900 cc of $H_2O$ at 85°. Allow to cool and dilute to 1 liter at 20°, as described under (a).

After the preparation of the soln proceed at once with the analysis.

**2**

### TOTAL SOLIDS

Thoroly mix the soln as prepared under **1**; pipet at once 100 cc into a weighed flat-bottomed glass dish, $2\frac{3}{4}$–3 inches in diameter; and (1) evaporate and dry for 16 hours in a combined evaporator and dryer[3] at 98–100°, or (2) evaporate on a steam bath and then dry for 12 hours on the bottom of a water oven at 98–100°. Remove immediately to a desiccator containing concentrated $H_2SO_4$ (place no more than 2 dishes in one desiccator) and weigh rapidly when cooled. Calculate the percentage of total solids.

### SOLUBLE SOLIDS

**3**

### PREPARATION OF FILTER

To about 75 cc of the soln, as prepared under **1**, add 1 g of kaolin. (The kaolin used should be neutral to phenolphthalein and should not yield more than 1 mg of soluble solids per 100 cc of filtrate of a 1% suspension in $H_2O$ after an hour's digestion at 20°.) Stir, and pour immediately into a single, 15 cm No. 590, S. & S. or No. 1F Swedish folded filter.[4] (These papers must be pleated by hand as they are not available in folded form.) Return the filtrate to the paper when approximately 25 cc has run thru and repeat the operation for an hour, thus transferring all the kaolin to the paper. At the end of an hour discard the soln on the filter by siphoning it off, disturbing the kaolin as little as possible. An ordinary wash bottle serves well for this purpose.

**4**

### DETERMINATION

Bring about 150 cc of the original soln, as prepared under **1**, to exactly 20°. Fill the filter, prepared as directed under **3**, with this soln and discard the filtrate until it runs thru clear. Keep the filter full, the temp. of the filtering soln at 20–25°, and the funnel and receiving vessel covered. Pipet at once 100 cc of the clear filtrate into a weighed dish, evaporate, and dry as directed under **2**. Calculate the percentage of soluble solids.

### 5 INSOLUBLE SOLIDS

The percentage of insoluble solids is the difference between **2** and **4.**

## NONTANNINS

### 6 REAGENT

*Hide powder.*[5]—This should be of woolly texture, well delimed, and 10 g of the water-free powder should require 12–13 cc of 0.1 *N* NaOH to neutralize it.

Calculate the quantity of air-dried hide powder that will be required for the number of determinations to be made, on the basis of 12.5 g of $H_2O$-free powder for each determination. Increase this calculated amount by 10 g of air-dried hide powder to provide a sufficient quantity for the determination of moisture in the wet chromed hide powder and also for a working leeway.

Digest the total quantity of air-dried hide powder with 10 times its weight of $H_2O$ until thoroly soaked. Then, for each g of the air-dried hide powder, so digested, add 1 cc of a 3% chrome alum soln ($K_2SO_4Cr_2(SO_4)_3.24H_2O$) and either agitate frequently for several hours and let stand overnight, or agitate in some form of mechanical shaker for an hour.[6] Transfer to a strong linen filter and squeeze thoroly; using the linen filter as a bag, leave the hide powder in it and digest for 15 min. with a quantity of water equal to 15 times the weight of the air-dried hide powder used. Filter, and squeeze to approximately 73% of $H_2O$, using a press if necessary. Very strong pressure is required to reduce the water content below 70%. Repeat the digestion and filtration 3 times. The wet chromed hide powder, as finally prepared, shall contain as nearly as possible 73% of $H_2O$. The moisture content must be not less than 72% nor more than 74%.[6] Determine moisture in 20 g of the squeezed hide powder, as directed under **2.**

### 7 DETERMINATION

Place 46 g of the wet hide powder, prepared as directed under **6,** in a shaker bottle of suitable capacity; add 200 cc of the tanning soln, prepared as directed under **1;** and shake immediately for 10 min. in a mechanical shaker. Squeeze at once thru linen; add 2 g of kaolin, as used under **3,** to the filtrate that contains the nontannins; stir; and filter thru a single, folded 18.5 cm filter paper (No. 1F Swedish preferred), refiltering until the filtrate is clear. Test the filtrate with gelatin-salt soln (1% gelatin and 10% salt), and if a precipitate forms, report the fact. Pipet 100 cc of the filtrate into a weighed dish and evaporate as directed under **2.** Correct the weight of the nontannin residue for the dilution caused by the $H_2O$ retained in the wet hide powder. Calculate the percentage of nontannins.

### 8 TANNIN

The percentage of tannin is the difference between **4** and **7.**

## SUGARS[7]

### 9 REAGENTS

The reagents and solns are described under **X, 10.**

### 10 PREPARATION OF SOLUTIONS

To 400 cc of soln as prepared under **1,** add 50 cc of saturated neutral Pb-acetate soln, shake well, and let stand for from 5 to 10 min. Filter thru a folded filter (18.5 cm), returning the filtrate until it is clear. Let the filter drain for about 30 min. after all the soln has been poured. Remove the excess Pb from the filtrate with dried $K_2HPO_4$, using the phosphate in the proportion of 5 g to 200 cc of the filtrate. (Measure the filtrate in a graduated cylinder. Usually from 360 to 380 cc will be obtained, requiring from 9 to 9.5 g of $K_2HPO_4$. Weigh the phosphate to within 0.1 g). After adding the phosphate, shake well for 4 or 5 min. and filter thru a folded filter (18.5 cm).

### 11 DETERMINATION

*Reducing sugars.*—Place 100 cc of the clarified deleaded soln obtained under **10** in a flask, add 33.3 cc of $H_2O$, and if the reduction is not made at once, 8–10 drops of toluene; shake well and stopper with a plug of cotton. Keep in a cool place and make the reduction within the following 24 hours. When ready for reduction, filter if toluene has been added. Determine reducing sugars in duplicate 50 cc aliquots, as directed under **XXXIV, 38.** After correcting the weight of the $Cu_2O$ for the blank of the Fehlings soln, find the equivalent milligrams of dextrose from **XLII, 9.** To express as percentage of dextrose, multiply the milligrams of dextrose by 3 and divide the result by grams of sample per liter of soln.

### 12

*Total sugars.*—Place 150 cc of the clarified, deleaded soln obtained under **10** in a 500 cc Erlenmeyer flask, add 7.5 cc of HCl (sp. gr. 1.18), and boil under a reflux condenser for exactly 1 hour. (If the soln foams at the start, which is unusual, add 5–10 drops of kerosene.) After boiling for an hour, remove the flask, stopper loosely when moderately cool, and let stand until ready for reduction, usually overnight. Cool the soln in ice $H_2O$ for from 20 to 30 min., add 2 drops of phenolphthalein soln, carefully neutralize with NaOH (1+1) and then add HCl dropwise until the color of the indicator is just discharged. After bringing the soln to room temp., transfer it to a 200 cc flask, make to mark, mix, and filter until clear. Reduce Fehling's soln with duplicate 50 cc aliquots and calculate results as directed under **11.**

### 13

*Non-reducing sugars.*—The percentage of non-reducing sugars is the difference between **11** and **12.**

## DETECTION OF SULFITE-CELLULOSE[8]

### 14 REAGENTS

*Sulfite-cellulose soln.*—Dissolve 0.5 g of the total solids derived from sulfite-cellulose in 1 liter of $H_2O$ and add sufficient tanning material, free from sulfite-cellulose, to give a concentration of 3.75–4.25 g of tannin per liter.

### 15 DETERMINATION

Place 5 cc of the tanning soln, prepared as directed under **1**, in a test tube. Add 0.5 cc of aniline and shake well; then add 2 cc of HCl and mix again. Compare the precipitate formed with that produced when the sulfite-cellulose soln is similarly treated. In the predetermined absence of the synthetic tanning material, Neradol-D, sulfite-cellulose is held to be present if the volume of the precipitate approximately equals or exceeds that of the comparison soln.

## LIQUORS

### 16 PREPARATION OF SOLUTION

Dilute the liquor with $H_2O$ at room temp. to contain approximately 0.7 g of solids in 100 cc of soln. If the liquor does not give a proper soln with $H_2O$ at room temp., it may be diluted with water at 80° and then cooled to 20°, as directed under **1 (a).**

### 17 TOTAL SOLIDS

Proceed as directed under **2**.

### 18 SOLUBLE SOLIDS

Proceed as directed under **4**.

### 19 NONTANNINS

Proceed as directed under **7**, using the quantity of wet chromed hide powder that will give the ratio between the tannin and hide powder shown in the following table:[9]

| TANNIN RANGE PER 100 cc | DRY HIDE POWDER PER 200 cc |
|---|---|
| *gram* | *gram* |
| 0.35–0.45 | 9.0–11.0 |
| 0.25–0.35 | 6.5– 9.0 |
| 0.15–0.25 | 4.0– 6.5 |
| 0.00–0.15 | 0.0– 4.0 |

## TOTAL ACIDITY

### 20 REAGENTS

(a) *Hematin.*—Digest 0.5 g of hematin in 100 cc of cold neutral 95% alcohol.

(b) *Gelatin soln.*—Soak 10 g of gelatine in $H_2O$ at room temp. for from 1 to 2 hours and then warm slightly, not exceeding 50°, to complete soln; add 25 cc of 95% alcohol, and dilute. If the gelatin soln is acid or alkaline, neutralize with 0.1 *N* NaOH or 0.1 *N* acetic acid, respectively, using hematin indicator, and dilute to 1 liter.

(c) *Kaolin.*—Digest with HCl (1+9), wash until it complies with the tests given under **3**, dry, and preserve in a tightly stoppered bottle.

(d) 0.1 *N sodium hydroxide* [cf. **II, 17 (c)**].

### 21 DETERMINATION[10]

Add 50 cc of the gelatin soln to 25 cc of the tanning liquor in a stoppered cylinder, dilute with $H_2O$ to 250 cc, add 15 g of the kaolin, and shake vigorously. Allow to settle for at least 15 min., remove 30 cc of the supernatant liquid, dilute with 50 cc of $H_2O$, and titrate with 0.1 *N* NaOH, using the hematin indicator. 1 cc of 0.1 *N* NaOH = 0.2% of acid, calculated as acetic, in the liquor.

## RAW AND SPENT MATERIALS

(Under raw materials are included woods, barks, leaves, etc.)

### 22 MOISTURE IN SAMPLE AS RECEIVED

Cut or break up large pieces and mix the sample rapidly to avoid change in moisture content. Dry as directed under **2**, a suitable weighed quantity, dependent upon the physical condition and moisture content of the sample.

### 23 PREPARATION OF SAMPLE

Dry the remainder of the sample at a temp. not above 60°, and grind to pass thru a 20-mesh sieve.

### 24 MOISTURE IN PREPARED SAMPLE

Take 10 g of the sample, prepared as directed under **23**; dry as directed under **2**; and calculate all results to an "as-received," "air-dried," or "moisture-free" basis, as desired.

## 25 EXTRACTION

(a) *Woods, barks, and spent materials.*—Weigh such a quantity of the sample as will give an extract containing as nearly as possible 4 g of tannin per liter. Transfer to a beaker and wet thoroly with hot $H_2O$. Place a perforated porcelain plate in a tin-lined Cu extractor of the general form shown in Fig. 11; on the plate place a layer of cotton, and wet thoroly with $H_2O$. Connect the extractor with an 800 cc Erlenmeyer flask (*G*) open the stopcock (*E*), and close the outlets (*C*) and (*D*). Pour into the extractor the material to be extracted, washing it into the extractor with hot $H_2O$. Return the percolate thru the extractor until it is practically clear. Place a layer of cotton on top of the material. Close the stopcock (*E*), connect with an 800 cc Erlenmeyer flask containing about 650 cc of $H_2O$, connect (*D*) by a delivery tube with a liter graduated collecting flask, return the total percolate to the extractor, and connect by means of the metal cap (*B*) with a block tin condenser (*A*) in such a way that the condensate will drip upon the layer of cotton. Boil the $H_2O$ in the flask and collect 400–500 cc of percolate from the side tube (*D*). Open the stopcock (*E*) and close the side tube (*D*); add $H_2O$ to the flask (*G*), if necessary, until it contains about 250 cc; and extract for 5 hours. Remove the extract, add 200 cc of $H_2O$ to the boiling flask, and continue the extraction for 9 hours. Thruout the extraction heat at such a rate that approximately 330 cc of $H_2O$ will be condensed per hour. Combine all the extracts in the graduated liter flask in which the first percolate was received. Heat to 80°, cool as directed under **1** (a), and dilute to the mark.

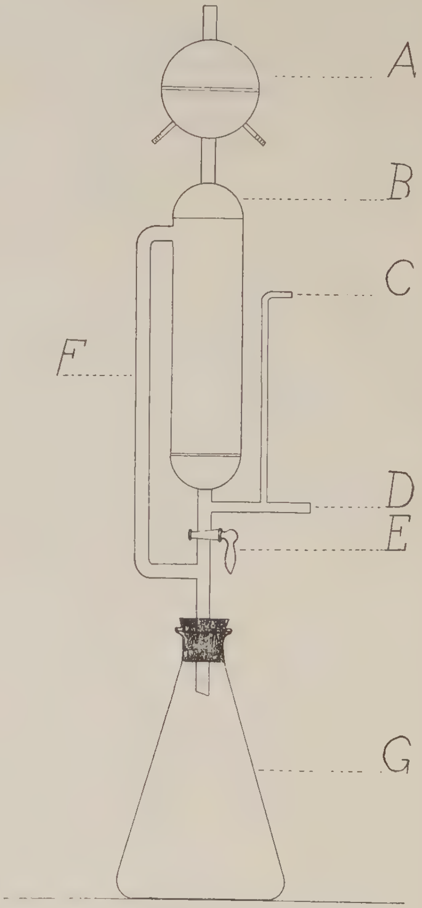

FIG. 11.—METAL EXTRACTOR USED FOR EXTRACTING TANNING MATERIALS

(b) *Materials other than woods, barks, and spent materials.*—Weigh, as directed under **25** (a), a charge sufficient to give 2 liters of extract containing 4 g of tannin per liter. Place in the extractor described under **25** (a), and digest for 1 hour with $H_2O$ at room temp., at the end of which period start the extractor. Keep stopcock (*E*) closed and collect entirely thru the side tube (*D*) 2 liters of percolate in approximately 7 hours. Heat the percolate to 80°, cool as directed under **1** (a), and dilute to the mark.

### 26 ANALYSIS OF THE EXTRACT

Proceed as directed under **2–8**, inclusive. With solns more dilute than specified, as is often the case with spent materials, reduce the quantity of hide powder used in the determination of nontannins in accordance with the concentration of the soln and the schedule given under **19**.

## SELECTED REFERENCES

[1] The proceedings of the A. O. A. C. that deal with the early development of the methods of analysis of tanning materials will be found in Bureau of Chemistry Bulletins Nos. 43, 47, 49, 51, 56, 62, 67, 73, 81, and 90. These have been assembled in J. Am. Leather Chem. Assoc. **15,** 1a–127 (1920).

[2] J. Am. Leather Chem. Assoc., **7,** 288, 296 (1912).

[3] Ibid., **1,** 32 (1906); **9,** 442 (1914).

[4] Ibid., **10**, 282 (1915).

[5] The official hide powder of the American Leather Chemists Association is prepared only by the Standard Manufacturing Company, Ridgway, Pa.

[6] J. Am. Leather Chem. Assoc., **7,** 292 (1912).

[7] Ibid., **23,** 91 (1928).

[8] Ibid., **9,** 36, 130 (1914).

[9] Ibid., **5,** 5 (1910).

[10] Ibid., **3,** 85 (1908); **4,** 194 (1909).

# XII. PLANTS

## 1 DIRECTIONS FOR SAMPLING[1]—TENTATIVE

When more than one plant is sampled, include in the sample a sufficient number of plants to insure that it represents adequately the average composition of the entire lot of plants sampled. This number cannot be stated definitely; it will depend upon the variability in composition of the plants. Details of the procedure must be determined by the purpose for which the sample is taken.

## 2 PREPARATION OF SAMPLE[1]—TENTATIVE

(a) *For mineral constituents.*—Thoroly remove all foreign matter from the material, especially adhering soil or sand, avoiding excessive washing to prevent leaching; air dry as rapidly as possible to prevent decomposition or loss in weight by respiration; grind; and preserve in tightly stoppered bottles. If the results are to be expressed on the fresh weight basis, record the weights of the sample before and after air drying. When determinations of Cu, Mn, Zn, Fe, Al, etc. are to be made, take precautions to prevent contamination of the sample by dust during air drying and from the grinding and sieving machinery.

(b) *For carbohydrates.*—Thoroly remove all foreign matter and rapidly grind or chop the material into fine pieces. Add the weighed sample to sufficient hot redistilled 95% alcohol to which sufficient precipitated $CaCO_3$ has been added to neutralize the acidity, using sufficient alcohol so that the final concentration, allowing for the water content of the sample, will be approximately 80%. Heat close to the boiling point on a steam or water bath for 30 min., stirring frequently. The samples may be stored until needed for analysis.

## 3 MOISTURE—TENTATIVE

Determine as directed under **XXVII, 2** or **6**.

## 4 ASH—TENTATIVE

Determine ash as directed under **XXVII, 8**.

## 5 SAND AND SILICA—OFFICIAL

Ignite 10–50 g* of the substance in a flat-bottomed Pt dish in a muffle, at a temp. not exceeding dull redness, until the residue is white or nearly so. Dissolve in HCl (1+4) and transfer to a beaker or evaporating dish; evaporate to dryness and heat on a water bath for 1 hour to render the $SiO_2$ insoluble. Moisten the residue with 5–10 cc of HCl, add about 50 cc of $H_2O$, heat on a water bath for a few min., filter thru a hardened filter, and wash thoroly. To this filtrate add the filtrate and washings from the soluble $SiO_2$ determination and dilute to 200 cc. Designate as A.

Wash the residue from the filter into a Pt dish and boil for about 5 min. with approximately 20 cc of a saturated soln of $Na_2CO_3$, add a few drops of a 10% NaOH soln, allow the mixture to settle, and decant thru an ignited and weighed Gooch crucible. Boil the residue in the dish with another 20 cc portion of the $Na_2CO_3$ soln and decant as before. Repeat the process. Transfer the residue to the Gooch crucible and wash thoroly, first with hot $H_2O$, then with a little HCl (1+4), and finally with hot $H_2O$ until free from chlorides. Dry the filter and contents, ignite, and weigh as sand. Confirm by microscopical examination.

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* "10–50 g" has been adopted as official, first action. The original method reads "10–20 g."

Determine the alkali soluble $SiO_2$ by the following procedure: Combine the alkaline filtrate and washings, acidify with HCl, evaporate to dryness, and dehydrate by heating to 110–120° for 2 hours. Moisten the residue with 5-10 cc of HCl, add about 50 cc of $H_2O$, heat on a water bath 10–15 min., filter thru an ashless filter or an ignited and weighed Gooch crucible, wash with hot $H_2O$, ignite, and weigh as $SiO_2$.

### FERRIC AND ALUMINUM OXIDES—OFFICIAL*

(Applicable to plant materials other than seeds.)

### 6 IRON AND ALUMINUM[2]—TENTATIVE

Take an aliquot of soln A containing approximately 40 mg of Fe- and $AlPO_4$. Oxidize the Fe. If the soln does not already contain an excess of phosphate, add to an aliquot containing approximately 40 mg of Fe- and $AlPO_4$, 0.5 g of $(NH_4)_2HPO_4$, stir until dissolved, and make up to 50 cc with distilled $H_2O$. Add a few drops of thymol blue and then add $NH_4OH$ until the soln just turns yellow. Run in 0.5 cc of HCl, follow with 25 cc of 25% $NH_4$-acetate, and stir. Let stand at room temp. until the precipitate settles (approximately 1 hour). Filter, and wash 10 times with hot 5% $NH_4NO_3$ soln. Ignite, and weigh as Fe-and $AlPO_4$.

Fuse the ignited precipitate in a Pt crucible with about 4 g of a mixture of equal parts of $Na_2CO_3$ and $K_2CO_3$. When the fusion is complete, allow the crucible to cool, add 5 cc of $H_2SO_4$, and heat until copious fumes of $SO_3$ are given off. Cool, transfer to a flask, add $H_2O$, and digest until the soln is clear. Reduce the Fe with Zn, cool, and titrate with 0.1 $N$ $KMnO_4$ soln (cf. **XXXVII, 58**). Report as percentage of $Fe_2O_3$. Calculate the oxide to phosphate and subtract from total Fe- and $AlPO_4$. This gives the $AlPO_4$. Calculate to and report as $Al_2O_3$.

### 7 MICRO METHOD FOR IRON ONLY[3]—TENTATIVE

Place an aliquot of soln A containing approximately 0.2 mg of Fe in a 50 cc volumetric flask. Oxidize the Fe by boiling with a few drops of $HNO_3$. Add $H_2O$ to make about 35 cc 5cc of HCl, and 0.3 cc of $HNO_3$, and cool to about 15°. Now add 10 cc of 20% KCNS soln, fill to the mark with distilled $H_2O$, and compare the intensity of color with that of a standard containing somewhere near the same amount of Fe as the sample and similarly prepared. If the quantity of Fe is extremely small, extract both the sample and standard with equal parts of amyl alcohol and ether and compare the extracts in a colorimeter. Calculate the amount of Fe present.

### 8 MICRO METHOD FOR ALUMINUM ONLY[4]—TENTATIVE

Take an aliquot of soln A containing approximately 0.05 mg of Al. Oxidize the Fe by boiling with a few drops of $HNO_3$ and transfer to a conical centrifuge tube of about 25 cc capacity with marks at 15, 20 and 25 cc. If the quantity of Fe is very small, add $Fe_2Cl_6$ soln equivalent to about 1 mg of iron; or if the quantity of phosphate present is small, add about 0.1 g of $(NH_4)_2HPO_4$ to insure complete precipitation of the Fe and Al. Dilute the contents to about 15 cc with distilled $H_2O$, neutralize with $NH_4OH$, using a drop of dilute methyl red as indicator, and add 1 cc of a saturated soln of $NH_4$-acetate. Place the tube in a water bath until the precipitate begins to settle (usually about 10 min.), centrifugalize, decant, and discard the supernatant liquid. Dissolve the precipitate in 1 cc of approximately 6 $N$ HCl with warming when necessary and dilute to 15 cc. Cool, add 1.25 cc of glacial acetic acid and 5 cc of 6 $N$ NaOH (special Al-free), wash down the sides, and fill to the 25 cc mark. Let stand for about 1 hour and centrifugalize. The precipitate contains the Fe and the soln the Al.

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* See note 7, p. xvii.

Transfer to a 50 cc volumetric flask as large an aliquot as can be drawn off. Add $H_2O$ to make about 20 cc, a small piece of litmus paper, and finally HCl (1+9) until the litmus paper just turns red. Determine the Al as follows: Add 5 cc of 5 *N* $NH_4$-acetate, 5 cc of 1.5 *N* HCl, and 2 cc of 0.1% of the dye Aluminon (ammonium salt of aurintricarboxylic acid) and place in a water bath at about 80° for 10 min. Add 5 cc of 5 *N* $NH_4Cl$, cool to room temp., add 5 cc of 3.2 *N* $(NH_4)_2CO_3$ while shaking gently, fill to the mark with distilled $H_2O$, and mix. At this point the reactions should be PH 7.1–7.3 and the red color of a blank should disappear in about 15 min. (The exact concentration of the reagents is not important, but the final pH is, and the amount of $NH_4CO_3$ necessary to bring the soln to the above pH should be determined by neutralizing similar solns without adding the dye.) Simultaneously with the above procedure run a standard (or standards if necessary) containing a given quantity of Al. After allowing the mixture to stand for 20 min. for the excess dye to decolorize, compare the color intensities and read the amount of Al from a curve plotted as described in the following paragraph.

If only a small number of determinations are to be made, prepare 4 standards containing 0.01, 0.03, 0.05 and 0.07 mg of Al, respectively, and run these with the samples. Compare all these solns with the standard containing 0.03 mg of Al and calculate the results to a colorimeter reading of 30 for this standard. Arbitrarily give 0.005 mg of Al a reading of 100 and with this and the 4 readings on the standards plot a curve.* Read the quantity of Al in each sample from this curve. If a large number of determinations are to be made extending over a period of time, it is advisable to make determinations on several series of standards and plot a curve from the average of these results. It is then necessary to run only 1 standard each time determinations are to be made, and the results can be read from the curve. Blanks must be run on both the Fe and Al determinations as nearly all the reagents contain traces of these elements.

## MANGANESE, CALCIUM, AND MAGNESIUM—OFFICIAL†

(Applicable to plant materials other than seeds.)

### 9 CALCIUM[5]—OFFICIAL, FIRST ACTION

Transfer an aliquot of soln A, under **5**, corresponding to 0.5–2 g of ash, to a 300 cc beaker and dilute to 200 cc. Add a few drops of alizarine or methyl orange indicator and make slightly alkaline with $NH_4OH$ (1+4). Add HCl (1+4) until the soln is just faintly acid, and then add 10 cc of 0.5 *N* HCl and 10 cc of a 2.5% oxalic acid soln. Boil the mixture and add, with constant stirring, 15 cc of a saturated soln of $(NH_4)_2$-oxalate. Continue to heat until the precipitate becomes granular. Cool; add, with constant stirring, 8 cc of a 20% Na-acetate soln; and allow to stand 12 hours. Filter, and wash with hot $H_2O$ until free from chlorides. Break the point of the filter with a stirring rod and wash the precipitate into the beaker with hot $H_2O$. Add 10 cc of $H_2SO_4$ (1+1), heat nearly to boiling, and titrate with 0.1 *N* $KMnO_4$ soln. Finally add the filter to the soln and complete the titration. 1 cc of 0.1 *N* $KMnO_4$ = 0.0028 g of CaO. Report as percentage of CaO.

Or proceed as follows: To the aliquot add 10 cc of a saturated soln of $NH_4$-oxalate and a drop of methyl red. Almost neutralize with $NH_2OH$, and boil until the precipitate is coarsely granular. Cool, add a few more drops of methyl red and then $NH_4OH$ (1+4) until the color is a faint pink (pH 5.0), and allow to stand at least 4 hours. Filter, and wash, etc., as directed above.

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* See original article, *J. Am. Chem. Soc.*, **51**, 2729 (1929).
† See note 7, p. xvii.

### *Micro Method*[6]*—Tentative*

### 10 REAGENTS

(a) *Potassium permanganate.*—0.02 *N*.
(b) *Ammonium oxalate.*—Saturated soln.
(c) *Glacial acetic acid.*—(1+1).
(d) *Ammonium hydroxide.*—(1+1).
(e) *Ammonium hydroxide.*—(1+49).
(f) *Sulfuric acid.*—(1+4).

### 11 APPARATUS

(a) *Centrifuge tubes.*—Conical tipped, about 15 cm long and of about 18 mm or 20 mm inside diameter.
(b) *Suction device.*—With tip as shown in diagram.
(c) *Centrifuge.*—About 2500 r.p.m.

### 12 DETERMINATION

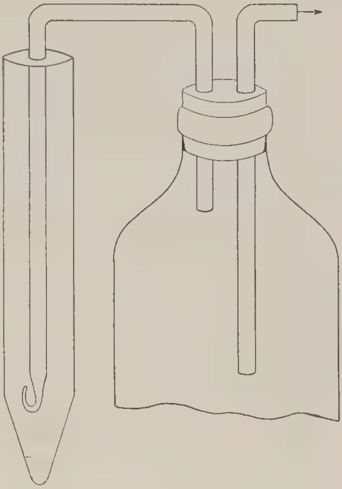

FIG. 12.—SUCTION DEVICE USED IN MICRO METHOD FOR THE DETERMINATION OF CALCIUM

Ignite 2 g of the substance in a small crucible in a muffle at dull red heat. Dissolve the ash in HCl (1+4) and transfer to a 100 cc beaker. Add 5 cc of HCl and evaporate to dryness on the steam bath to dehydrate the $SiO_2$. Moisten the residue with 5 cc of HCl, add about 50 cc of distilled $H_2O$, heat for a few minutes on the steam bath, transfer to a 100 cc volumetric flask, cool quickly to room temp., make to volume, shake, and filter, discarding the first portion of the filtrate. Pipet a 15 cc aliquot into a conical-tipped centrifuge tube containing 2 cc of saturated $(NH_4)$-oxalate soln and 2 drops of 0.05% methyl red. Add 2 cc of dilute acetic acid, rotating the tube to mix its contents thoroly. Add, while intermittently rotating the tube, $NH_4OH$ (1+4) until the soln is faintly alkaline, after which add a few drops of dilute acetic acid with a dropper until the color is adjusted to a faint pink (pH 5.0). (It is important at this point to rotate the tube so that the last bit of liquid in the conical tip is the color required.) Allow the mixture to stand at least 4 hours and whirl the tube in the centrifuge for 15 min. (The precipitate should then be in a firm lump in the tip of the tube.) Remove the supernatant liquid by means of the suction device shown in the diagram, taking care not to disturb the precipitate. Wash the precipitate by adding 2 cc of Reagent (e), rotating the tube to break up the precipitate. (It may be necessary to jar the tube sharply.) Return the tube to the centrifuge for 10 min., and again remove the supernatant liquid and wash with the reagent as before. Repeat this operation until the precipitate has been washed 3 times. When the supernatant liquid has been removed after the final centrifugalizing add 2 cc of Reagent (f) to the tube, break up the precipitate as before, heat on the steam bath to between 80 and 90°, and titrate in the tube with 0.02 *N* $KMnO_4$, rotating the liquid during the titration to attain a proper end point. If the tube cools below 60° during the addition of the $KMnO_4$, reheat it in the steam bath for a few minutes and complete the titration. Run a blank on an identical amount of dilute $H_2SO_4$ in a similar tube heated to the same temp. to determine the quantity of 0.02 $N$ $KMnO_4$ necessary to give the color of the end point. Subtract this value from the buret reading. 1 cc of 0.02 $N$ $KMnO_4 = 0.0004$ g of Ca. Report as percentage of Ca.

### 13

### MAGNESIUM[7]—OFFICIAL, FIRST ACTION

To the combined filtrate and washings from the Ca determination (9) add 40 cc of $HNO_3$ and evaporate to dryness to decompose the $NH_4$ salts. Take up with HCl (1+4) and make to a volume of about 100 cc with $H_2O$. Add 5 cc of a 10% Na-citrate soln and 10 cc of a 10% $Na_2H$-$PO_4$ soln, or enough to precipitate all the Mg. Add $NH_4OH$ (1+4), with constant stirring, until the soln is faintly alkaline; then add about 25 cc of $NH_4OH$ and allow to stand in a cool place overnight. Filter and wash with $NH_4OH$ (1+9). Dissolve the precipitate in HCl (1+4) and reprecipitate as before. Allow to stand several hours, filter, wash free of chlorides with the $NH_4OH$ (1+9), ignite, and weigh as $Mg_2P_2O_7$. Calculate and report the result as percentage of MgO.

### 14

### MANGANESE[8]—OFFICIAL

To an aliquot of soln A, under 5, representing about 0.2-0.5 g of ash, add 15 cc of concentrated $H_2SO_4$ and evaporate to about 30 cc. Add 5-10 cc of $HNO_3$ and continue the evaporation. (It is neither necessary nor advisable to evaporate until dense fumes appear, since the $Fe(SO_4)_3$ then dissolves with difficulty. $HNO_3$ may be present, but not HCl.) Add $H_2O$, a little at a time; heat until the Fe salts have dissolved; and dilute to about 150 cc. Add 0.3 g of $KIO_4$, boil for a few minutes or until the color of the $KMnO_4$ shows no further increase in intensity, and allow to cool.

Prepare the standard in the following manner: To a volume of $H_2O$ equal to the sample add 15 cc of $H_2SO_4$ and sufficient pure $Fe(NO_3)_3$, free from Mn to equal approximately the quantity of Fe in the sample. Add a measured quantity of 0.1 $N$ $KMnO_4$ soln until the color is slightly darker than the sample, and then add 0.3 g of $KIO_4$ and boil for a few minutes. When cool, transfer the sample and standard to 250 cc flasks and dilute to the mark with $H_2O$. If the color is weak, it may be necessary to dilute to less than 250 cc. Compare the colors in any standard colorimeter. Calculate and report the results as percentage of $Mn_3O_4$.

## SODIUM AND POTASSIUM

### 15

### *Method I—Official*

Saturate 10 g of the sample with $H_2SO_4$ and ignite in a muffle at a low red heat to destroy the organic matter. Heat the residue on a steam or water bath with 5–10 cc of HCl and about 50 cc of $H_2O$. Transfer to a beaker and add $NH_4OH$ dropwise until the precipitate formed requires several seconds to dissolve, thus leaving the soln but faintly acid. Heat nearly to boiling, and add $NH_4OH$ to precipitate all the Fe, Al, etc. Boil in a covered beaker for about 1 min.; remove; and if no $NH_3$ is detected by smelling continue the addition, dropwise, until it can be detected. Do not allow the precipitate to settle, but stir and pour on the filter. Wash immediately with hot $H_2O$, using, to effect rapid filtration, a fine jet directed around the edge of the precipitate to cut it free from the paper. Wash the precipitate several times, return it to the original beaker, dissolve with a few drops of HCl, and warm. Reprecipitate the Fe, Al, and $P_2O_5$ with $NH_4OH$ as directed above; filter; and wash until free from chlorides. Evaporate the combined filtrates and washings to dryness, heat below redness until $NH_4$-salts are expelled, and dissolve in hot $H_2O$. Add 5 cc of a saturated soln of $Ba(OH)_2$, heat to boiling, allow to settle a few min., and determine whether or not the precipitation is complete by the addition of more of the $Ba(OH)_2$ soln to a little of the clear liquid. When no further precipitate is produced, filter and wash thoroly with hot $H_2O$. Heat the filtrate to boiling and add $NH_4OH$ (1+4) and a 10% $(NH_4)_2CO_3$ soln to complete the precipitation of the Ba, Ca, etc. Let stand a short time on a water bath, filter, and wash the precipitate thoroly with hot $H_2O$. Evaporate the filtrate and washings to dryness; expel $NH_4$-salts by heating below redness; treat with a little hot $H_2O$; and add a few drops of the dilute $NH_4OH$, 1 or 2 drops of the $(NH_4)_2CO_3$ soln, and a few drops of a saturated soln of $NH_4$-oxalate. Let stand for a few min. on a water bath and set aside for a few hours. Filter, evaporate to complete dryness on a water bath, and heat at a temp. not exceeding dull redness until all $NH_4$-salts are expelled and the residue is nearly or quite white. Dissolve in a minimum quantity of $H_2O$, filter into a weighed Pt dish, add a few drops of HCl, evaporate to dryness on a water bath, heat at a temp. not exceeding dull redness, cool in a desiccator, and weigh as KCl plus NaCl. Repeat the heating until constant weight is obtained. Dissolve in a small quantity of $H_2O$; if any residue remains, repeat the separation until the residue of KCl and NaCl is entirely soluble. Dissolve the residue with $H_2O$, add an excess of Pt soln [cf. **II, 41 (b)**], and proceed as directed under **II, 46.**

### 16 *Method II—Official*

Proceed as directed under **15** thru "Let stand a short time on a water bath," the point at which the Ba, Ca, etc., have been precipitated with $NH_4OH$ and $(NH_4)_2CO_3$, and then proceed as follows:

Filter into a beaker, add 1 or 2 drops of HCl (1+4) and 1 cc of $(NH_4)_2SO_4$ soln (75 g to 1 liter), digest several hours on a water bath, and filter into a weighed Pt dish. Evaporate to dryness, heat to full redness, and add 1 g of powdered $(Na_4)_2CO_3$. Heat to expel excess of $(NH_4)_2CO_3$, cool, and weigh the sulfates of Na and K. Determine K as directed under **II, 43 (a)**.

## COPPER[9]—OFFICIAL, FIRST ACTION

### 17 REAGENTS

(a) *Potassium ethyl xanthate.*—0.1% water soln prepared fresh each time it is used.

(b) *Standard copper sulfate.*—Dissolve 0.3928 g of pure $CuSO_4 . 5H_2O$ in $H_2O$, dilute in a volumetric flask to 1000 cc, and mix. 1 cc = 0.0001 g of Cu.

(c) *Filter-paper pulp.*—Moisten and tear a good grade of sheet filter paper into bits and place in a porcelain dish of the proper size. Add, while stirring with a glass rod, enough cold HCl to disintegrate the paper and reduce the mass to a mushy consistency. Transfer the pulp to a large Büchner funnel and wash free of acid, using suction. Transfer the washed pulp into a clean glass bottle and add $H_2O$ to make a thick pulp suitable for making a pad in a Caldwell crucible. (The precipitates of Cu and Zn obtained in these methods can be readily filtered and washed upon pads made with this filter-paper pulp by the use of the suction pump.)

(d) *Hydrochloric acid.*—0.25 *M* soln.

### 18 DETERMINATION

Ash 100–500 g of the finely divided air-dried plant material in $SiO_2$ dishes with a small flame, but do not allow the plant material to burn with a blaze. After the volatile matter has been dispelled, complete the ashing in a muffle furnace maintained at the temp. of a faint red glow. Hasten the ashing process by removing the dishes from the muffle at intervals and breaking up the lumps with a Pt stirring rod. After the C has been oxidized as completely as possible, cool the dish, moisten the ash with $H_2O$, wash into a 250 cc beaker, and cover with a watch-glass. Decompose the ash with HCl (1+1) introduced thru the lip of the beaker beneath the watch-glass by means of a pipet. After effervescence has ceased, rinse the watch-glass into the beaker, filter the insoluble residue out on a Büchner funnel, and wash free of chlorides. If the insoluble residue contains undecomposed particles of C, transfer it into a $SiO_2$ dish and reignite in the muffle furnace until all particles of C are decomposed and a light colored ash remains. Redigest the ash on a hot water bath with 15 cc of HCl (1+1), filter, and wash free of chlorides. Combine the filtrates in a clean porcelain dish, evaporate to dryness, and bake at 110° until the HCl is expelled. Moisten the dry residue with 10 cc of HCl (1+1) and digest, with stirring, for 10 min. Dilute with hot $H_2O$, filter out the $SiO_2$, wash free of chlorides, combine with the insoluble residue, ignite, and weigh. Make the filtrate to about 250 cc, heat to near the boiling point, and pass a slow stream of $H_2S$ thru the soln for 15 min. Rinse the $H_2S$ delivery tube into the flask, tightly stopper, and set aside until the precipitate settles and the supernatant soln is clear. Filter the CuS off on a pad of paper pulp and wash with Reagent (**d**) saturated with $H_2S$; ignite in a porcelain crucible and dissolve the CuO in a few drops of $HNO_3$ (1+9) and one drop of HCl (1+9). Filter the soln and wash the filter paper clean. Evaporate the soln to dryness in a porcelain dish 3 times with the addition of a few drops of $HNO_3$ (1+9) and take up with a very small drop of $HNO_3$ (1+9) delivered from a stirring rod having a sharp point. Make to a volume of 50 cc, and transfer an aliquot of 5 cc to a Nessler tube containing 10 cc of Reagent (**a**). Mix the solns and dilute to 25 cc. Transfer 10 cc of Reagent (**a**) to a second Nessler tube, dilute to a volume of about 15 cc, and add the standard Cu soln (**b**) dropwise with thoro mixing with a glass stirring rod until the color in the standard tube apparently matches the color in the tube containing the sample. Make the volume and the final adjustment of the color of the standard in the tube containing it. Record the number of cc of the standard Cu soln required and calculate the percentage of Cu.

To the filtrate from the CuS add 5 cc of $HNO_3$ (1+1) and boil for 10 min. to oxidize the remaining metals. Cool the soln and make to a convenient volume (250 cc). From this stock soln take suitable aliquots for the determination of Zn or other elements contained in the ash of the sample.

### ZINC[9]—OFFICIAL, FIRST ACTION

**19** REAGENTS

(**a**) *Potassium ferrocyanide.*—2 g per 100 cc of freshly prepared soln.

(**b**) *Zinc sulfate.*—Dissolve 1 g of C. P. Zn in $H_2SO_4$ and dilute to 1000 cc. 1 cc = 0.001 g of Zn.

**20** DETERMINATION

Transfer an aliquot equivalent to 25 g of plant material from the stock soln (filtrate from the Cu determination—last paragraph under **18**) to a 250 cc Erlenmeyer flask and add $NH_4OH$ in slight excess. Dissolve the precipitate in a slight excess of pure acetic acid, saturate the soln with $H_2S$, and set aside for several hours for the precipitate to settle. [The acidity of the soln must be kept between pH 2 and pH 3, and the presence of a citrate helps to prevent the precipitation of Fe and Mn. Hence at this point add about 2 g of citric acid, ammonia until neutral to methyl orange, and then 10 cc of a formic soln (containing in 100 cc of the mixture, 3 cc of $NH_4OH$, 20 cc of 90% formic acid, and 25 g of $(NH_4)_2SO_4$).] Filter on a pad of paper pulp and wash with a dilute soln of acetic acid containing $NH_4$-acetate saturated with $H_2S$. Ignite the pad of paper pulp and precipitate in a porcelain crucible, cool, dissolve the residue in a few drops of HCl (1+9) and warm on the hot water bath. Transfer to a 100 cc beaker and add $NH_4OH$ in slight excess. Heat on the water bath for 5 min., filter, and wash the precipitate. Add acetic acid in slight excess to the filtrate and saturate the hot soln with $H_2S$; stopper tightly and set aside several hours in a warm place for the precipitate to settle; filter; wash as previously described and ignite in a porcelain crucible. Dissolve the ignited residue of ZnO in 10 cc of 0.1 *N* $H_2SO_4$, make to a volume of 50 cc, and mix. Transfer an aliquot of 5 cc to a 50 cc Nessler tube containing 5 cc of Reagent (**a**). Dilute to 50 cc, mix with a stirring rod, and let stand 5–10 min. To another Nessler tube containing 5 cc of Reagent (**a**) diluted to about 40 cc, add dropwise with stirring the standard soln (**b**) until the turbidity in the standard matches the turbidity of the sample. From the number of cc of the Zn standard required calculate the percentage of Zn contained in the sample.

## ARSENIC—TENTATIVE

### 21 PREPARATION OF SOLUTION

Prepare as directed under **XXIX, 3.**

### 22 DETERMINATION

Determine the As as directed under **XXIX, 4,** or take an aliquot and determine as directed under **VI, 13,** beginning with "add 3 cc of $H_2SO_4$."

## SULFUR

### *Sodium Peroxide Method*[10]*—Official*

### 23 PREPARATION OF SOLUTION

Place 1.5–2.5 g of material in a Ni crucible of about 100 cc capacity and add 5 g of anhydrous $Na_2CO_3$. Mix thoroly, using a Ni or Pt rod, and moisten with approximately 2 cc of $H_2O$. Add $Na_2O_2$, approximately 0.5 g at a time, thoroly mixing the charge after each addition, and continue until the mixture becomes nearly dry and quite granular. (Usually about 5 g of $Na_2O_2$ is required.) Place the crucible over a low alcohol or other S-free flame and heat carefully, with occasional stirring, until the contents are fused. (If the material ignites, the determination is worthless.) After fusion, remove the crucible, allow to cool somewhat, and cover the hardened mass with more of the $Na_2O_2$ to a depth of about 0.5 cm. Heat gradually and finally with full flame until fusion again takes place, rotating the crucible from time to time in order to bring any particles adhering to the sides into contact with the oxiding material. Continue the heating for 10 min. after fusion is complete. Cool somewhat, place the warm crucible and contents in a 600 cc beaker, and carefully add about 100 cc of $H_2O$. After the initial violent action has ceased, wash the material out of the crucible, make slightly acid with HCl (adding small portions at a time), transfer to a 500 cc flask, cool, and dilute to volume. Filter, and determine sulfates in an aliquot of the filtrate as directed under **25**.

### 24 REAGENT

*Barium chloride.—10%* soln.

### 25 DETERMINATION

Heat to boiling and add slowly in small quantities a 10% $BaCl_2$ soln until no further precipitate is formed. Continue the boiling for about 5 min. and allow to stand for 5 hours or longer in a warm place. Decant the liquid thru an ashless filter or an ignited and weighed Gooch crucible, treat the precipitate with 15–20 cc of boiling $H_2O$, transfer to the filter, and wash with boiling $H_2O$ until the filtrate is free from chlorides. Dry the precipitate and filter, ignite, and weigh as $BaSO_4$. Multiply the result by the factor 0.13736 and report as percentage of S.

### *Magnesium Nitrate Method*[11]—*Official, First Action*

#### 26 PREPARATION OF SOLUTION

Weigh a quantity of the sample sufficient to give a precipitate of not less than 30 mg of $BaSO_4$ into a large porcelain or Sillimanite crucible. Add 7.5 cc of $Mg(NO_3)_2$ soln [**II, 5**(e)], taking care that all the material is brought in contact with the soln, and heat on an electric hot plate (180°) until no further action takes place. Transfer the crucible while hot to an electric muffle and allow it to remain at low heat (muffle must not show any red) until the charge is thoroly oxidized. No black particles should remain. (It may be necessary to break up the charge and return to the muffle.) Remove the crucible from the muffle and allow to cool. Add $H_2O$, then HCl in excess. Bring the soln to a boil, filter, and wash thoroly. If preferred, transfer the soln to a 250 cc volumetric flask before filtering and make to the mark with $H_2O$.

#### 27 DETERMINATION

Dilute the entire filtered soln, prepared as directed under **26**, to 200 cc or take an aliquot of 100 cc of the measured volume, make to 200 cc, and proceed as directed under **25**.

### PHOSPHORUS[11]

#### 28 *Method I—Official, first action*

Take 100 cc of the original soln prepared as directed under **26**, or evaporate the filtrate and washings from the S determination, **27**, to 75 cc and proceed as directed under **II, 7** or **10**.

### *II. Micro Method*[12]—*Tentative*

#### 29 REAGENTS

(a) *Potassium dihydrogen phosphate standard.*—Dissolve 0.4394 g of pure dry $KH_2PO_4$ in distilled $H_2O$ and make up to a liter. 50 cc of this soln diluted to 200 cc gives a standard of which 2 cc = 0.05 mg of P.

(b) *Ammonium molybdate.*—Dissolve 25 g of $NH_4$-molybdate in 300 cc of $H_2O$. Dilute 75 cc of $H_2SO_4$ to 200 cc and add to the $NH_4$-molybdate soln.

(c) *Hydroquinone.*—Dissolve 0.5 g of hydroquinone in 100 cc of distilled $H_2O$, and add one drop of concentrated $H_2SO_4$ to retard oxidation.

(d) *Sodium sulfite.*—Dissolve 200 g of $Na_2SO_3$ in distilled $H_2O$, make up to a liter, and filter. Either keep this soln well-stoppered or make it up fresh each time.

(e) *Magnesium nitrate.*—Dissolve 160 g of MgO in $HNO_3$ (1+1), avoiding an excess of the acid; add a little MgO in excess, boil, filter from the excess MgO, $Fe_2O_3$, etc., and dilute to 1 liter.

#### 30 PREPARATION OF SOLUTION

To 1 or 2 g of the substance in a small Sillimanite crucible add 1 cc of the $Mg(NO_3)_2$ soln and place on the steam bath. After a few min. cautiously add a few drops of HCl, taking care that the formation of gas bubbles does not push portions of the sample over the edge of the crucible. Make 2 or 3 further additions of a few drops of HCl while the sample is on the bath so that as it approaches dryness there is a tendency for it to char. If the contents of the crucible become so viscous that no further drying may be obtained on the bath, complete the drying on a hot plate, put on a crucible cover, transfer to a cold muffle, and ignite at dull red heat for 6 hours or until an even grey ash is obtained. It may be necessary to cool the crucible, dissolve the ash in a little $H_2O$ or alcoholic glycerol, evaporate to dryness, and return uncovered to the muffle for another 4 or 5 hours. Cool, take up with HCl (1 +4), and transfer to a 100 cc beaker. Add 5 cc of HCl and evaporate to dryness on the steam bath to dehydrate the $SiO_2$. Moisten the residue with 2 cc of HCl, add about 50 cc of distilled $H_2O$, heat for a few minutes on the bath, transfer to a 100 cc volumetric flask, cool immediately, make to volume, and filter, discarding the first portion of the filtrate.

### 31 DETERMINATION

To a 5 cc aliquot of the filtrate in a 10 cc volumetric flask add 1 cc of $NH_4$-molybdate, rotate the flask to mix, and allow to stand a few moments. Add 1 cc of hydroquinone, again rotate the flask, and add 1 cc of $Na_2SO_3$. These last 3 additions may be made with a Mohr pipet. Make to volume with distilled $H_2O$, stopper the mouth of the flask with the thumb or forefinger, and shake to mix the contents thoroly. Allow to stand 30 min. and compare immediately in a colorimeter with 2 cc of the standard $KH_2PO_4$ soln treated simultaneously and in an identical manner. With either the unknown or standard set at 25.0 mm, readings within 10 mm (i.e. a range of 20 mm) are accurate. If the concentration of P in the unknown set is outside this range, it may be brought nearer to that of the standard by diluting the filtrate, ashing a smaller or larger sample, making the filtrate to a smaller or larger volume, or using a smaller aliquot. Report as percentage of P.

## CHLORINE—OFFICIAL

### 32 PREPARATION OF SOLUTION

Moisten 5 g of the substance in a Pt dish with 20 cc of a 5% $Na_2CO_3$ soln, evaporate to dryness, and ignite as thoroly as possible at a temp. not exceeding dull redness. Extract with hot $H_2O$, filter, and wash. Return the residue to the Pt dish and ignite to an ash; dissolve in $HNO_3$ (1 +4), filter from any insoluble residue, wash thoroly, and add this soln to the $H_2O$ extract.

### 33 *I. Gravimetric Method*

To the soln prepared as directed in **32** add a 10% $AgNO_3$ soln, avoiding more than a slight excess. Heat to boiling, protect from the light, and allow to stand until the precipitate is granular. Filter on a weighed Gooch crucible, previously heated to 140–150°, and wash with hot $H_2O$, testing the filtrate to prove excess of $AgNO_3$. Dry the AgCl at 140–150°, cool, and weigh. Report as percentage of Cl.

### *II. Volumetric Method*[13]

### 34 REAGENTS

(a) *Silver nitrate.*—Adjust to exact 0.1 *N* strength by standardizing against a 0.1 *N* NaCl soln containing 5.846 g of pure NaCl per liter.

(b) *Ammonium or potassium thiocyanate.*—0.1 *N*. Adjust by titrating against the 0.1 *N* $AgNO_3$.

(c) *Ferric indicator.*—A saturated soln of ferric ammonium alum.

(d) *Nitric acid.*—Free from lower oxides of N by diluting the usual pure acid with about ¼ volume of $H_2O$, and boiling until perfectly colorless.

### 35 DETERMINATION

To the soln prepared as directed under **32**, add a known volume of the 0.1 *N* $AgNO_3$ in slight excess. Stir well, filter, and wash the AgCl precipitate thoroly. To the combined filtrate and washings add 5 cc of the ferric indicator and a few cc of the $HNO_3$ and titrate the excess of Ag with the 0.1 *N* thiocyanate until a permanent light brown color appears. From the number of cc of 0.1 *N* $AgNO_3$ used, calculate the quantity of Cl. 1 cc of 0.1 *N* $AgNO_3=0.00355$ g of Cl.

### 36 TOTAL NITROGEN

Determine as directed under **II, 27.**

## SUGARS[14]—TENTATIVE

### 37 PREPARATION OF SOLUTION

*Extraction.*—Prepare the sample as described under **2(b)**. Pour the alcoholic soln thru a filter paper or extraction thimble, catching the filtrate in a volumetric flask. Transfer the insoluble material to a beaker, cover with 80% alcohol, warm on a steam bath for 1 hour, allow to cool, and again pour the alcoholic soln thru the same filter. If the second filtrate is highly colored, repeat the extraction. Transfer the residue to the filter, allow to drain, and dry. Grind the residue so that all the particles will pass thru a 1-mm sieve, then transfer it to an extraction thimble and extract for 12 hours in a Soxhlet apparatus with 80% alcohol. Dry the residue and save for the starch determination. Combine the alcoholic filtrates and make to volume at a definite temp. with 80% alcohol.

*Clearing.*—Place an aliquot of the alcoholic extract in a beaker on the steam bath and drive off the alcohol. Avoid evaporation to dryness by adding $H_2O$ if necessary. When the odor of alcohol has disappeared from the sample, add about 100 cc of distilled $H_2O$ and heat to 80° to soften gummy precipitates and break up insoluble masses. Cool to room temp. and proceed as directed under **(a)** or **(b)**.

**(a)** Transfer the soln to a volumetric flask and rinse the beaker thoroly with $H_2O$, adding the rinsings to the contents of the flask. Add enough saturated neutral Pb-acetate to produce a flocculent precipitate, shake thoroly, and allow to stand 15 min. Test the supernatant liquid with a few drops of saturated Pb-acetate. If more precipitate forms, shake and allow to stand again; if no further precipitate forms, dilute to the mark with $H_2O$, mix thoroly, and filter thru a dry filter. Add sufficient solid Na-oxalate to the filtrate to precipitate all the Pb, and refilter thru a dry paper. Test the filtrate for the presence of Pb with a little solid Na-oxalate.

**(b)** Add double the minimum amount of saturated neutral Pb-acetate soln that is required to cause complete precipitation, as found by testing a portion of the supernatant liquid with a few drops of dilute Na-oxalate soln. After allowing the mixture to stand a few min. only, filter immediately into a beaker to which has been added an estimated excess of Na-oxalate crystals. Allow the Pb precipitate to drain on the filter and wash with cold $H_2O$ until the filtrate no longer gives a precipitate in the oxalate soln. Excess of oxalate must be assured by testing with a drop of dilute Pb-acetate soln. Filter off and wash the precipitated Pb-oxalate, catching the filtrate and washings in a volumetric flask. Dilute to the mark with $H_2O$ and mix.

## REDUCING SUGARS—TENTATIVE

### 39 *I. Munson and Walker General Method*

Proceed as directed under **XXXIV, 37.**

### *II. Quisumbing and Thomas Method*[15]

### 40 REAGENTS

**(a)** *Copper sulfate soln.*—Wash crystals of C. P. $CuSO_4.5H_2O$ free from dust, etc. with distilled $H_2O$, dissolve in hot $H_2O$ to make a saturated soln, and filter. Determine the Cu electrolytically and dilute the soln so that 25 cc of it will contain 525 mg of Cu or 41.2 g of $CuSO_4$-pentahydrate in 500 cc of soln.

(b) *Alkaline tartrate soln.*—Prepare a saturated soln of NaOH (purified by alcohol) and let stand until the insoluble carbonates and other impurities have settled out several days. Siphon off the clear soln and establish its alkalinity by titration with standard acid. Dissolve 173 g of highest purity Rochelle salts in $H_2O$ in a 500 cc graduated flask and add the calculated quantity of NaOH soln so that 500 cc of this alkaline tartrate soln will contain exactly 65 g of NaOH. Make to the mark with $H_2O$.

### **41** PRECIPITATION OF CUPROUS OXIDE

Measure exactly 25 cc each of the $CuSO_4$ and alkaline tartrate solns into a 400 cc Pyrex or Bohemian glass beaker, the diameter of which is about 9 cm. Add 50 cc of sugar soln containing preferably 50–150 mg of sugar. Cover the beaker with a watch-glass and place the beaker in a water bath which is maintained at 80°. After exactly 30 min. digestion filter the $Cu_2O$ by suction thru a mat of asbestos in a Gooch crucible. Wash the precipitate with $H_2O$. Determine the Cu by one of the methods below. Calculate the weight of sugar from the tables of Quisumbing and Thomas (**XLII, 17**).

## DETERMINATION OF REDUCED COPPER

### **42** *I. Direct Weighing of Cuprous Oxide*

Proceed as directed under **XXXIV, 39.**

### **43** *II. Volumetric Permanganate Method*

(a) Proceed as directed under **XXXIV, 42.**

(b) Filter and wash the $Cu_2O$ as directed in **41**. Transfer the asbestos film to the beaker, suspend in $H_2O$, and heat the precipitate and asbestos thoroly. Rinse the crucible and the lip of the beaker with 10 cc of a soln made by dissolving 240.9 g of crystalline ferric ammonium sulfate and 200 cc of $H_2SO_4$ in $H_2O$ to 1 liter. (Note: Cool the diluted $H_2SO_4$ before dissolving the alum.) Receive the rinsings in the beaker containing the precipitate. Wash the crucible and sides of the beaker with boiling $H_2O$, catching the washings in the beaker. Stir until all the Cu is dissolved. A green soln is obtained. Titrate at once with continual stirring with $KMnO_4$ soln until the pink due to the $KMnO_4$ persists for about 10–15 seconds. 1 cc of 0.05 $N$ $KMnO_4 = 0.0031785$ g of Cu. Standardize the $KMnO_4$ as follows: Dry overnight about 0.66 g of Na-oxalate (pure) in a weighing tube in an oven at 100° and carefully weigh off 3 samples of 0.10–0.15 g each. Dissolve each sample in 100 cc of $H_2O$, add 5 cc of $H_2SO_4$ $(1+1)$, warm to 70°, and titrate the $KMnO_4$ against this soln. Take the average of the 3 titrations. 1 cc of 0.05 $N$ $KMnO_4 = 0.00335$ g of Na-oxalate.

### **44** *III. Electrolytic Deposition from Sulfuric and Nitric Acid Solutions*

Proceed as directed under **XXXIV, 43.**

### **45** SUCROSE—TENTATIVE

(a) *Hydrochloric acid inversion.*—Proceed as directed under **XXVII, 22.**

(b) When glucosides which are easily hydrolyzed by HCl are present, sucrose may be inverted by invertase. The preparation and use of invertase are described under **XXXIV, 21.** The quantity of invertase to be used depends on its activity. Avoid a large excess because it causes difficulty in the filtration of the reduced Cu.

### STARCH[14]—TENTATIVE

**46** *Diastase Method with Subsequent Acid Hydrolysis*

Proceed as directed under **XXVII, 25.** If the sample has been previously extracted in a Soxhlet with hot alcohol, further extraction with alcohol and ether is unnecessary.

### 47 ETHER EXTRACT—TENTATIVE

Proceed as directed under **XXVII, 15.**

### 48 CRUDE FIBER—TENTATIVE

Proceed as directed under **XXVII, 19.**

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[1] J. Assoc. Official Agr. Chem., **13**, 224 (1930).
[2] Ibid., **11**, 203 (1928). Modified.
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[10] U. S. Dept. Agr. Bur. Chem. Bull., **105**, p. 151; **116**, p. 92; **137**, p. 30.
[11] J. Assoc. Official Agr. Chem., **6**, 415 (1923).
[12] Ibid., **14**, 216 (1931); J. Biol. Chem., **59**, 255 (1929).
[13] Sutton, Systematic Handbook of Volumetric Analysis, 16th Ed., 1911, p. 146.
[14] J. Assoc. Official Agr. Chem., **14**, 225 (1931).
[15] J. Am. Chem. Soc., **43**, 1503 (1921).

# XIII. FIBERS*

# XIV. PAPER AND PAPER MATERIALS*

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* See note at bottom of p. xv.

# XV. BAKING POWDERS AND BAKING CHEMICALS

### 1 PREPARATION OF SAMPLE—OFFICIAL

Remove the entire sample from the package, pass thru a 20-mesh sieve, and mix thoroly.

## TOTAL CARBON DIOXIDE

## GRAVIMETRIC METHODS

### *I. Using Knorr's Apparatus*[1]*—Official*

### 2 REAGENTS

(**a**) *Potassium hydroxide soln.*—Dissolve 25 g of KOH in 50 cc of $H_2O$.
(**b**) *Soda lime.*—Finely granulate and free from dust by sifting.
(**c**) *Sulfuric acid.*—Concentrated.
(**d**) *Sulfuric acid.*—(1+5).

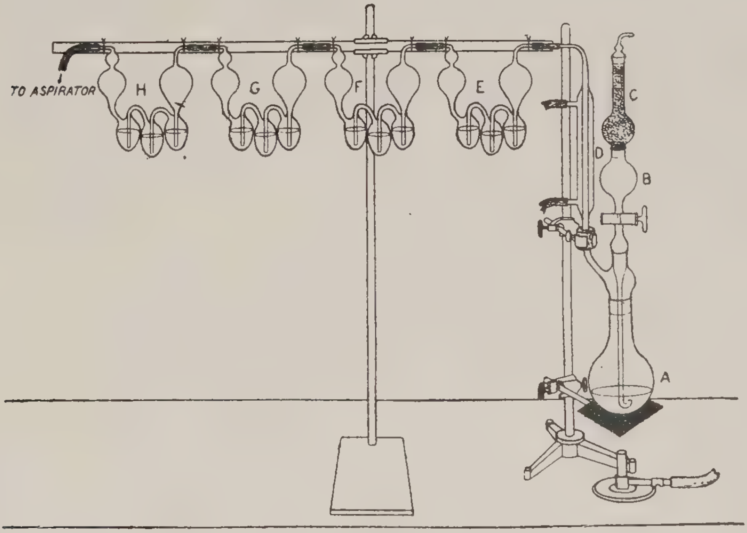

FIG. 13.—KNORR'S APPARATUS FOR THE DETERMINATION OF CARBON DIOXIDE

### 3 APPARATUS

Connect a flask (*A*) by means of a ground-glass joint with a glass connection thru the upper part of which passes a dropping funnel (*B*), and join at the side with a Liebig condenser (*D*). Connect the mouth of the funnel *B* by means of a perforated stopper with a soda lime tube (*C*). Connect the upper end of condenser *D* by a rubber joint with a Geissler bulb (*E*), containing concentrated $H_2SO_4$ for drying the gas passing into the next Geissler bulb (*F*), which contains the strong KOH soln. Then connect bulb *F* with a third Geissler bulb (*G*), containing concentrated $H_2SO_4$ for the absorption of moisture escaping from (*F*) and to a fourth Geissler bulb (*H*), also containing $H_2SO_4$, as a precaution to prevent moisture from the air being absorbed by (*G*). Connect *H* with an aspirator. Many analysts prefer to replace the bulb *F* by two U-tubes filled with sifted soda lime.

### 4 DETERMINATION

Place 0.5–2 g of the sample, the quantity depending upon the percentage of $CO_2$ present, in flask *A*, which must be perfectly dry. Close the flask with the stopper which carries the funnel tube and the tube connecting with the absorption apparatus. Weigh separately *F* and *G* and attach them to the apparatus. If two soda lime tubes are employed, weigh them separately and refill the first when the second increases materially in weight. Nearly fill the funnel tube *B* with sulfuric acid (1+5) and place the soda lime tube *C* in position. Aspirate air thru the Geissler bulbs at a rate of about two bubbles per second. Open the stopper of the funnel and allow the acid to run slowly into the flask, taking care that the evolution of gas is so gradual as not materially to increase the current thru the Geissler bulbs. After all the acid has been introduced, close the stopcock in *B*, continue the aspiration, and gradually heat the contents of the flask to boiling. While the flask is being heated the aspirator tube may be removed, altho when using ground-glass joints many analysts prefer to aspirate during the entire operation. Continue the boiling for a few min. after the water has begun to condense in *D*; then remove the flame, open the stopcock in tube *B*, and continue aspiration while the apparatus cools. Remove bulbs *F* and *G* and weigh. The increase in weight is due to $CO_2$.

### *II. Using Heidenhain's Apparatus*[2]*—Official*

(Extremely accurate but too complicated for routine work.)

### 5 REAGENTS

(**a**) *Calcium chloride.*—Use $CaCl_2$ dehydrated at 200°, but not fused. Grind coarsely and sift thru No. 18 wire gauze to remove the extremely coarse, and thru No. 30 wire gauze to remove the very fine, particles.

(**b**) *Soda lime.*—Grind and sift the soda lime for the absorption tubes in the manner described under (**a**). The soda lime should not be too dry, as it must not absorb moisture to a greater degree than the $CaCl_2$.

### 6 APPARATUS

Fill cylinder *A* with soda lime to remove $CO_2$ from the air passing thru the apparatus. A thick layer of cotton at the upper end prevents soda lime dust from being carried over. Connect the cylinder *A* by means of a perforated rubber stopper and a bent glass tube having a stopcock (*B*) and a capillary constriction (*C*) with a short piece of rubber tubing to which is attached a short piece of glass tubing (*E*), fitted with a perforated rubber stopper. The stopper fits tightly into the constriction of *D*, the funnel of which is cylindrical in shape, $\frac{3}{4}$ inch in diameter at the upper end, $\frac{3}{8}$ inch at the lower end, and 4 inches long. The stem of *D* passes thru a doubly perforated rubber stopper and extends almost to the bottom of the evolution flask (*F*), which is ordinarily of 150 cc capacity but, in the case of foaming liquids, may hold 300 cc. Thru the second perforation in the stopper connect flask *F* with a reflux condenser (*G*). To the upper end of the condenser attach a U-tube containing a little $CaCl_2$ (to be renewed when it has liquefied) to retain the bulk of the moisture. Connect this U-tube with a second U-tube (*H*), filled with coarse $CaCl_2$, and this in turn with a third U-tube (*K*) filled at *I* with a 3-inch column of pumice stone impregnated with $CuSO_4$ and completely dehydrated at 150°, the remainder of the tube being filled with fine $CaCl_2$. Connect the U-tube *K* with a bent glass tube having a stopcock ($L$), which is closed when the apparatus is not in use. Next attach the absorption U-tubes ($M$) and ($N$), which are ½ inch in diameter and 5 inches long, the first filled mainly with soda lime but containing a little $CaCl_2$ at the end where the air current enters, the second filled one-half with soda lime and one-half with $CaCl_2$, the $CaCl_2$ being placed at the side where the air current leaves. Connect $N$ with a guard tube ($O$), filled with $CaCl_2$ on the side toward $N$ and with soda lime on the side toward $P$, the latter being a small U-tube trapped with glycerol to indicate the passage of the air current. Connect $P$ with a safety bottle ($R$), to receive any water which may be sucked back from the aspirator, and connect $R$ with a 4-liter aspirator bottle ($S$).

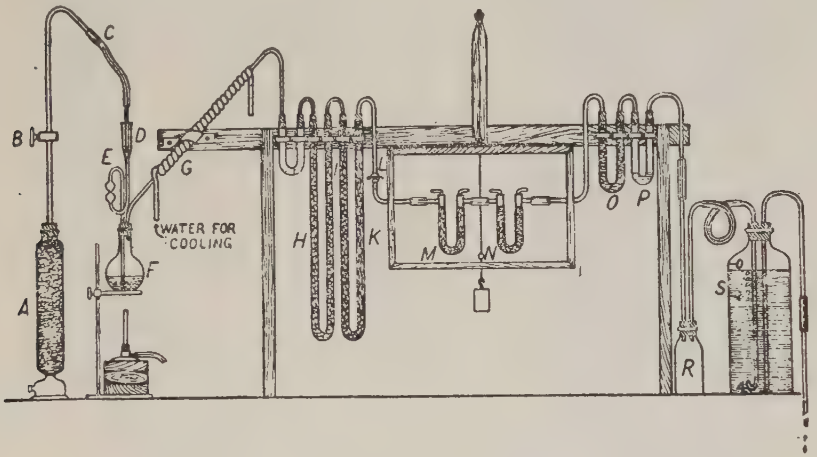

FIG. 14.—HEIDENHAIN'S APPARATUS FOR THE DETERMINATION OF CARBON DIOXIDE

The tubes $M$ and $N$ should hold about 20 g, making the capacity of $M$ for $CO_2$ almost 1 g and that of $N$ for moisture 0.2 g. $M$ should be refilled when its weight has increased 0.75 g and $N$ after increase of 0.1 g in weight. Use the best grade of rubber for all connections, applying a trace of castor oil as a lubricant. For connections of the weighed tubes use rubber tubing boiled in weak lye, washed, and dried. Also apply a little castor oil, wiping it off thoroly before connecting the tubing. Before using the apparatus fill $H$ and $K$ with $CO_2$ in order to saturate the $CaCl_2$ with the gas and exhaust after several hours.

### 7 DETERMINATION

To find the allowable rapidity of the air current used during the determination, proceed as follows: Charge the apparatus exactly as for an analysis, leaving out the carbonate. Begin to aspirate at the rate of about 50 cc per min. After 2 liters has been aspirated, weigh the tubes $M$ and $N$. If they have lost weight, repeat the experiment with 40 cc per min., and continue until the weight of the tubes remains constant. If the work has been done cautiously, the first tube will show a loss equal to the gain of the second tube. Do not exceed the safe speed thus found.

Shortly before weighing open $M$ and $N$ for a moment to allow equalization of air, then weigh at the air temp. of the balance room and note the thermometer and barometer readings. Connect the tubes with the apparatus and test the tightness of the joints by closing $A$ at the bottom, opening all the cocks, starting the aspirator, and observing *P*, in which the liquid should soon come to a standstill. Then disconnect the aspirator, close *B*, and remove *F*. Put the sample into *P*, using about 1 g of $Na_2CO_3$ or $CaCO_3$ or about 2 g of baking powder; connect *F*, and start condenser *G*. Introduce 50 cc of HCl (1+2.5) thru *D*, lifting *E* slightly and allowing only small quantities of the dilute acid to enter at a time. Light the burner under *F*, heat to boiling, and then reduce the flame to keep the liquid just at the boiling point. If no more air passes *P*, start the aspiration. When the water stops running from *S*, open *B* carefully and adjust the outflow of the aspirator by raising or lowering the syphon to one-half the safe speed.

After *M* has cooled, increase the current to the full safe speed and aspirate 3 liters, continuing boiling to the end of the aspiration. After the tubes have reached the temp. of the balance room, open for a moment and weigh. When extreme accuracy is desired, note again the thermometer and barometer readings and apply correction to the following formula:

$-(A^2-A^1)\times T$ and $+(B^2-B^1)\times B$, in which
$A^1$ = the temperature at first weighing in degrees C;
$A^2$ = the temperature at second weighing in degrees C;
$B^1$ = the barometric pressure at first weighing in mm; and
$B^2$ = the barometric pressure at second weighing in mm.

*T* and *B* are constants found from the following formulas:

$T = V \times 0.0000039$ g;
$B = V \times 0.0000015$ g, in which
0.0000039 = change in weight of 1 cc of air for 1°;
0.0000015 = change in weight of 1 cc of air for 1 mm pressure;

and the value of *V* is obtained from

$$V=\frac{G}{2.7}+\frac{F}{2.0}-\frac{G+F}{8.5},$$

representing the differential volume affected by temperature and pressure and being a constant for the tubes, and in which

$G$ = the weight of the empty tubes;
$F$ = the weight of the fillings;
2.7 = the specific gravity of glass;
2.0 = the specific gravity of filling;
8.5 = the specific gravity of brass;
$\frac{G}{2.7}+\frac{F}{2.0}$ = volume of tubes and fillings; and
$\frac{G+F}{8.5}$ = volume of brass weights.

### *Gasometric Method*[3]*—Official*

#### 8 REAGENTS

(a) *Sulfuric acid.*—(1+5).

(b) *Displacement soln.*—Dissolve 100 g of NaCl or $Na_2SO_4.10H_2O$ in 350 cc of $H_2O$. Add approximately 1 g of $NaHCO_3$ and 2 cc of methyl orange indicator [**VI, 3** (f)] and then sufficient $H_2SO_4$ (1+5) to make just acid (a decided pink color). Stir until all $CO_2$ is removed. This soln is used in the gas-measuring tube and leveling bulb and seldom needs to be replaced.

### 9 APPARATUS

Connect a decomposition flask (*A*) by means of a glass T-tube (*B*), provided with a stopcock (*C*), to a graduated gas-measuring tube (*D*), which in turn is connected with a leveling bulb (*E*). For *A* always use a 250 cc wide-mouthed extraction flask of Pyrex or other resistant glass fitted with a two-holed rubber stopper, thru one hole of which passes the extended tip of a 25 cc buret (*F*) and thru the other a glass tube of the same diameter as the connecting T-tube. Use a buret graduated in cc at 20°, numbered at 5 cc intervals, and provided with an extra long tip bent to pass thru the rubber stopper. Connect the glass tube leading from the decomposition flask to the T-tube by means of rubber tubing to permit rotation of the flask. Use a gas-measuring tube graduated in cc at 20°, the zero mark being placed at a point 25 cc below the top marking to allow for graduating upwards from 0 to 25 cc and downward from 0 to 200 cc. By means of a long rubber tube connect the gas-measuring tube with the leveling bulb, which has a capacity of about 300 cc.

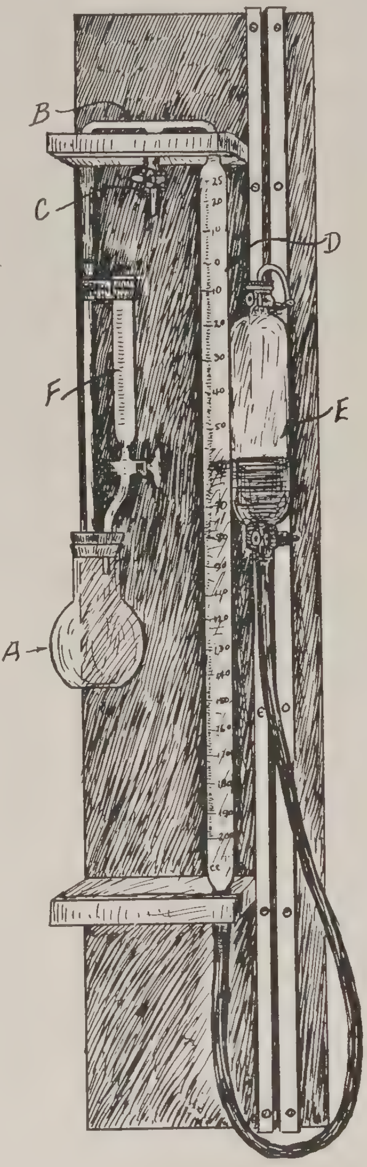

FIG. 15.—APPARATUS FOR THE GASOMETRIC DETERMINATION OF CARBON DIOXIDE

### 10 DETERMINATION[4]

Weigh 1.7 g of the sample, prepared as directed under **1**, into flask *A* and connect this flask with the apparatus (Fig. 15). Open stopcock *C* and by means of the leveling bulb *E* bring the displacement soln to the 10 cc graduation above the zero mark. (This 10 cc is practically equal in volume to the volume of acid to be used in the decomposition.) Allow the apparatus to stand 1–2 min. to insure that the temp. and pressure within the apparatus are the same as those of the room. Close the stopcock, lower the leveling bulb somewhat to reduce the pressure within the apparatus, and slowly run into the decomposition flask from buret *F* 10 cc of the dilute $H_2SO_4$. To prevent the liberated $CO_2$ from escaping thru the acid buret into the air, keep the displacement soln in the leveling bulb at all times during the decomposition at a lower level than that in the gas-measuring tube. Rotate and then vigorously agitate the decomposition flask to secure intimate mixture of the contents. Allow to stand for 5 min. to secure equilibrium. Equalize the pressure in the measuring tube by means of the leveling bulb and read the volume of gas in the tube. Observe the temp. of the air surrounding the apparatus and also the barometric pressure at the time and multiply the number of cc of gas evolved by the factor given in the table for this temp. and pressure. Divide the corrected reading by 10 to obtain the percentage by weight of $CO_2$ in the sample.

### RESIDUAL CARBON DIOXIDE[5]

#### 11 *Gravimetric Method—Official*

Weigh 2 g of the sample, prepared as directed under **1**, into a flask suitable for the subsequent determination of $CO_2$; add 20 cc of cold $H_2O$; and allow to stand 20 min. Place the flask in a metal drying cell surrounded by boiling $H_2O$ and heat, with occasional shaking, for 20 min. To complete the reaction and drive off the last traces of gas from the semi-solid mass, heat quickly to boiling and boil for 1 min. Aspirate until the air in the flask is thoroly changed, and determine the residual $CO_2$ by absorption, as directed under **4** or **7**.

#### 12 *Gasometric Method[6]—Official*

(See Table **24**, correction factors for the gasometric determination of $CO_2$.)

Place 1.7 g of the sample, prepared as directed under **1**, in the decomposition flask, **9** (*A*); add 20 cc of $H_2O$ and allow to stand 20 min. Place the flask in a metal drying cell surrounded by boiling $H_2O$ and heat, with occasional shaking, for 20 min. To complete the reaction, heat quickly to boiling and boil for 1 min. Cool to room temp. connect the flask to the apparatus described under **9**, and determine the $CO_2$ present by treating with 10 cc of the dilute $H_2SO_4$ as directed under **10**. To prevent foaming add 1–3 drops of caprylic alcohol to the baking powder in the decomposition flask.

### 13 AVAILABLE CARBON DIOXIDE—OFFICIAL

Subtract the residual $CO_2$ from the total $CO_2$.

### NEUTRALIZING VALUE

#### 14 *I. Of Acid-Reacting Materials Other Than Phosphates—Official*

Dissolve 1 g of the sample in hot $H_2O$ and titrate with 0.2 *N* NaOH, using phenolphthalein indicator.

#### 15 *II. Of Monocalcium Phosphate[7]—Tentative*

Weigh 0.84 g of monocalcium phosphate into a small beaker or casserole, add 25 cc of $H_2O$ and 5 drops of a 0.2% soln of phenolphthalein[8], and titrate with 0.2 *N* NaOH, to a faint pink. Heat to boiling, boil 1 min., and again continue the titration, while the soln is hot, to a faint pink color, adding the bulk of the standard alkali soln rapidly and with vigorous stirring. The total buret reading multiplied by 2 equals the neutralizing strength of 100 parts of phosphate in terms of $NaHCO_3$.

### 16 TARTARIC ACID, FREE OR COMBINED (QUALITATIVE TEST)[9]—TENTATIVE

(Applicable in the presence of phosphates.)

Shake repeatedly about 5 g of the sample with about 250 cc of cold $H_2O$ in a flask and allow the insoluble portion to subside. Decant the soln thru a filter and evaporate the filtrate to dryness. Powder the residue, add a few drops of 1% resorcin soln and about 3 cc of $H_2SO_4$, and heat slowly. Tartaric acid is indicated by a rose-red color, which is discharged on dilution with water.

## 17 TOTAL TARTARIC ACID—OFFICIAL

(Applicable only in the absence of Al salts, Ca salts, and phosphates.)

Into a 500 cc porcelain casserole or similarly shaped dish weigh 1.88 g of the sample, prepared as directed under **1**. Add 10 cc of $H_2O$ and then 10 cc of HCl (1+1) cautiously to avoid loss due to the sudden evolution of $CO_2$. Heat gently until most of the starch present is hydrolized. Add slowly and with constant stirring 15 cc of $K_2CO_3$ soln containing 328 g of $K_2CO_3$ per liter, boil gently on a hot plate for 1 min., and evaporate on a steam bath to incipient crystallization. Remove from the steam bath and add, dropwise with constant stirring, 3 cc of glacial acetic acid. Add 2 cc more of the glacial acetic acid and continue the stirring for 3 min. Add 150 cc of 95% alcohol, carefully rinsing down the sides of the dish with the alcohol; stir vigorously for 5 min.; and let stand for at least 1 hour. Decant thru a Gooch crucible containing a thin layer of paper pulp or thru filter paper on a perforated disk, and wash, largely by decantation, with 95% alcohol until the combined filtrate and washings measure 550 cc. Test the last few cc of filtrate with dilute litmus tincture to be sure the precipitate has been properly washed. Return the paper pulp, or disk, containing a part of the precipitate to the residue in the porcelain dish and add 120 cc of hot $H_2O$. Add sufficient 0.2 *N* alkali soln to neutralize most, but not all, of the acidity. Boil the soln for 5 min. and complete the titration with 0.2 *N* alkali, using phenolphthalein indicator. The number of cc of 0.2 *N* alkali times 2 equals the percentage of K-bitartrate to which 0.15% is to be added to compensate for loss due to solubility. 1 cc of 0.2 *N* alkali = 0.02641 g of tartaric anhydride, 0.03001 g of tartaric acid, and 0.03763 g of K-bitartrate.

## 18 FREE TARTARIC ACID (QUALITATIVE TEST)—OFFICIAL

Extract 5 g of the sample with absolute alcohol and evaporate the alcohol from the extract. Dissolve the residue in dilute $NH_4OH$, transfer to a test tube, add a good sized crystal of $AgNO_3$, and heat gently. Tartaric acid is indicated by the formation of a silver mirror. If desired, the absolute alcoholic extract may be tested as directed under **16.**

## STARCH

### 19 *I. Direct Inversion Method—Official*

(For baking powders and baking chemicals free from calcium.)

Weigh 5 g of the powder into a 500 cc volumetric flask and proceed as directed under **XXVII, 23.**

### 20 *II. Indirect Method*[10]*—Official*

(For baking powders and baking chemicals containing calcium.)

Mix 5 g of the powder with 200 cc of HCl (1+11) in a 500 cc volumetric flask and allow the mixture to stand for an hour, shaking frequently. Filter on an 11 cm hardened filter, taking care to obtain a clear filtrate. Rinse the flask once without attempting to remove all the starch, and wash the paper twice with cold $H_2O$. Carefully wash the starch from the paper back into the flask with 200 cc of $H_2O$. Add 20 cc of HCl (sp. gr. 1.125) and proceed as directed under **XXVII, 23.** The treatment with dilute hydrochloric acid (1+11), without dissolving the starch, removes effectively the Ca, which otherwise would be precipitated as tartrate by the alkaline Cu soln.

### 21 *III. Modified McGill Method—Tentative*

Digest 1 g of the powder with 150 cc of HCl (1+11) for 24 hours at room temp., with occasional shaking. Filter on a Gooch crucible, wash thoroly with cold $H_2O$ and then once with alcohol and once with ether. Dry at 110° (4 hours is usually sufficient), cool, and weigh. Burn off the starch, weigh again, and determine the starch by difference. The results by this method on cream of tartar powders and tartaric acid powders agree closely with those obtained by Cu reduction. The results on other types of baking powders are usually satisfactory, but in some instances they may be over 2% too high.

## ALUMINUM

### *I. Qualitative Test—Official**

### *II. Qualitative Test[11]—Tentative*

(In presence of phosphates.)

#### 22 REAGENTS

(a) *Hydrochloric acid.*—Approximately normal soln. Dilute 9 cc of HCl to 100 cc.

(b) *Ammonium acetate soln.*—3 *N*. Dissolve 23.1 g of $NH_4$-acetate in $H_2O$ and dilute to 100 cc.

(c) *Aurintricarboxylic acid.*—0.1% soln. Dissolve 1 g in $H_2O$ and dilute to 100 cc.

#### 23 DETECTION

Dissolve 1-5 g of the baking powder in 5 cc of *N* HCl and 5 cc of 3 *N* $NH_4$-acetate. Add 5 cc of a 0.1% soln of aurintricarboxylic acid, mix, and allow the lake formation to take place. Make the soln alkaline with $NH_4OH$ containing a small quantity of $(NH_4)_2CO_3$. A bright persistent red precipitate indicates the presence of Al.

### *III. By precipitation with Phenylhydrazine[12]—Tentative*

#### 24 REAGENTS

(a) *Ammonium bisulfite soln.*—Pass $SO_2$ into a cool soln of $NH_4OH$ (1+1) until the color of the soln becomes distinctly yellow.

(b) *Phenylhydrazine bisulfite soln.*—To a few cc of phenylhydrazine add gradually a saturated soln of $SO_2$ until the precipitate of phenylhydrazine sulfite, which at first separates out in crystals, is almost redissolved. If the precipitate is completely dissolved, add a drop or two of phenylhydrazine until a slight precipitate is obtained. Filter the soln before using. (From 5-10 cc of this soln in 100 cc of $H_2O$ is sufficient strength for washing the $Al(OH)_3$ precipitate. If well stoppered, this concentrated soln of phenylhydrazine bisulfite will keep indefinitely.)

(c) *Dilute hydrochloric acid.*—Add 10 volumes of $H_2O$ to 4 of strong HCl.

#### 25 DETERMINATION

Ignite 3 g of baking powder at a temp. not exceeding 550°. As soon as the C has burned off, take up the residue in HCl (4+10) and boil gently to assist soln. Filter into a 300 cc volumetric flask and wash with hot $H_2O$. Ignite the insoluble residue and filter paper in a Pt crucible and fuse the residue with about 2 g of $Na_2CO_3$. Dissolve the fused mass in water and HCl and transfer to the volumetric flask containing the original filtrate. Cool, and make up to volume.

Transfer 100 cc aliquots to 400 cc beakers. Heat nearly to boiling, add dilute $NH_4OH$ until a slight permanent precipitate forms, then just redissolve this precipitate with a drop or two of dilute HCl. Add, dropwise with constant stirring, 10 or 12 drops of a saturated soln of $NH_4HSO_3$. Then add to the hot soln sufficient phenylhydrazine to precipitate the $Al(OH)_3$ completely. (1 or 2 cc is generally enough; an excess colors the soln yellow.) If a permanent precipitate does not form at this point, add dilute $NH_4OH$ carefully, dropwise, just to a permanent precipitate, and then complete the precipitation by adding a few more drops of the phenylhydrazine. Let stand a few min. for the precipitate to settle, then filter while still warm. Wash the precipitate with warm $H_2O$ containing the phenylhydrazine bisulfite until the washings give no test for iron when yellow $NH_4$-sulfite is added.

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* See Note 7, p. xvii.

Place the filter paper containing the precipitate in a weighed Pt crucible. Dry, char, and ignite at a low temp. After the filter paper has completely burned, continue the ignition at a bright red heat to constant weight. Weigh quickly with the cover on the crucible as the precipitate is very hydroscopic. A second weighing is always necessary. The precipitate consists of $Al_2O_3$ and Al-phosphate.

Fuse the ignited precipitate with about 2 g of $Na_2CO_3$ and dissolve the fusion in $HNO_3$ (1+9). Transfer to a 250 cc beaker and boil to insure that all the phosphoric acid is in the ortho state. Cool. Transfer to a 200 cc flask, make up to volume, and use 50 cc aliquots to determine the $P_2O_5$. Multiply the weight of $P_2O_5$ obtained by 4 and subtract the product from the weight of combined precipitates obtained above. The difference is the weight of $Al_2O_3$ in 1 g of baking powder.

Weight $Al_2O_3 \times 100$ = percentage of $Al_2O_3$.
Percentage of $Al_2O_3 \times 4.749$ = percentage of $Na_2Al_2(SO_4)_4$.

If the baking powder contains a significant quantity of $SiO_2$, remove it by evaporating the HCl soln of the powder to dryness and dehydrating at 105° for 2 hours. Add to the dry mass 10 cc of HCl and 100 cc of $H_2O$, boil, filter off the $SiO_2$, and proceed as in **24**.

## Ash[13]

### 26 INSOLUBLE ASH AND PREPARATION OF SOLUTION—OFFICIAL

Char 5 g of the sample in a Pt dish at a heat below redness. Boil the carbonaceous mass with HCl (1+2.5), filter into a 500 cc volumetric flask, and wash with hot $H_2O$. Return the residue, together with the paper, to the Pt dish, and burn to a white ash. Boil again with the dilute HCl, filter, wash, unite the two filtrates, and dilute to 500 cc. Incinerate the residue after the last filtration and weigh the ash insoluble in acid.

### 27 IRON AND ALUMINUM—OFFICIAL

Draw a 100 cc aliquot of the soln, prepared as directed under **26**, and separate $SiO_2$ if necessary. Mix the soln with 10% Na-phosphate soln in excess. Add strong $NH_4OH$ until a permanent precipitate is obtained, then HCl, dropwise, until the precipitate is dissolved. Bring the soln to a boil and boil for 2–3 min.; mix with a considerable excess of 50% $NH_4$-acetate soln and 4 cc of 80% acetic acid. As soon as the precipitate of Al-phosphate, mixed with Fe-phosphate, has settled, collect on a filter, wash with hot $H_2O$, ignite, and weigh. Fuse the mixed phosphates with 10 parts of $Na_2CO_3$, dissolve in $H_2SO_4$ (1+6), reduce with zinc, and determine the iron by titration with a standard permanganate soln (**XXXVII, 58**). In the same soln determine the phosphoric acid, as directed under **II, 7** or **10**. To obtain the weight of $Al_2O_3$, subtract the sum of the weights of $Fe_2O_3$ and $P_2O_5$ from the weight of the mixed phosphates.

### 28 CALCIUM—OFFICIAL

Heat the combined filtrate and washings obtained under **27** to 50° and add an excess of saturated $NH_4$-oxalate soln. Allow to stand in a warm place until the precipitate has settled, filter, wash the precipitate with hot $H_2O$, dry, and ignite over a Bunsen burner and finally over a blast lamp. Cool in a desiccator and weigh as CaO.

### 29 POTASSIUM AND SODIUM—OFFICIAL

Evaporate an aliquot of the soln, prepared as directed under **26**, nearly to dryness to remove the excess of HCl, dilute, and heat to boiling. While still boiling add 10% $BaCl_2$ soln as long as a precipitate forms and then enough saturated $Ba(OH)_2$ soln to make the liquid strongly alkaline. As soon as the precipitate has settled, filter and wash with hot $H_2O$; heat the filtrate to boiling; add sufficient $(NH_4)_2CO_3$ [1 part of $(NH_4)_2CO_3$ in 5 of $NH_4OH$ soln (1+12)] to precipitate all the Ba, filter, and wash with hot $H_2O$. Evaporate the filtrate to dryness and ignite the residue below redness to remove $NH_4$-salts. Add to the residue a little $H_2O$ and a few drops of the $(NH_4)_2CO_3$ soln. Filter into a weighed Pt dish, evaporate, ignite below redness, and weigh the mixed K- and Na-chlorides. Determine K in the mixed chlorides as directed under **II, 46**, beginning with "Digest the residue with hot $H_2O$, filter thru a small filter." Calculate the K so found to its equivalent of KCl and subtract this result from the weight of the mixed chlorides to obtain the weight of NaCl.

### 30 PHOSPHORIC ACID—OFFICIAL

Mix 5 g of the sample with a little $Mg(NO_3)_2$ soln, dry, ignite, dissolve in HCl (1+2.5), and dilute the soln to a definite volume. In an aliquot of the soln determine phosphoric acid as directed under **II, 7** or **10.**

### 31 SULFURIC ACID[14]—OFFICIAL

Boil 5 g of the sample for 1.5 hours with a mixture of 300 cc of $H_2O$ and 15 cc of HCl. Filter, wash filter thoroly with hot $H_2O$, cool the combined filtrate and washings, and dilute to a volume of 500 cc. Determine $H_2SO_4$ in an aliquot of 100 cc as directed under **XII, 25.**

### 32 AMMONIA—OFFICIAL

Introduce 2 g of the sample into a distillation flask, add 300–400 cc of $H_2O$ and an excess of NaOH soln (1+1), connect with a condenser, and distil into a measured volume of standard acid. Titrate the excess of acid in the distillate with standard alkali, using methyl red or cochineal indicator.

## LEAD

### *I. Electrolytic Method[15]—Official*

### 33 REAGENTS

(a) *Hydrochloric acid.*—Dilute 1 volume of HCl with 2 volumes of $H_2O$.
(b) *Nitric acid.*—Dilute $HNO_3$ with an equal volume of $H_2O$.
(c) *Acetic acid.*—Dilute 1 volume of acetic acid with 4 volumes of $H_2O$.
(d) *Potassium dichromate soln.*—A saturated water soln of $K_2Cr_2O_7$.

### 34 APPARATUS

(a) *Direct current.*—The potential should be constant and not less than 6 volts. A 3-cell, 6-volt storage battery will suffice.

(b) *Voltmeter.*

(c) *Ammeter.*—Capacity 1.0 ampere and graduated to 0.01 ampere.

(d) *Rheostat.*—Not less than 100 ohms resistance.

(e) *Anode.*—Weld a piece of Pt foil 25 mm square to a small piece of Pt wire, the end of which is sealed into a glass tube and extends into the tube for a distance of 1 cm. The Pt foil extends vertically downward from the glass tube. Have the glass tube of sufficient diameter to admit a column of Hg and a contact wire and long enough, about 6 inches, to admit of considerable adjustment. Make a contact with the Pt electrode by partly filling the glass tube with Hg and inserting the wire from the circuit. Use special care to see that the Hg does not leak around the fused-in wire.

(f) *Cathode.*—Use 30 cm of No. 22 Pt wire, one end of which is sealed into a glass tube in the same manner as the anode (e); shape the remainder of the wire into a spiral of about the same width as the anode and extend it downward from the glass tube parallel to the anode.

(g) *Stirring apparatus.*—The electrolyte may be kept in constant motion by a glass paddle mounted on a glass rod driven by a motor.

### 35 DETERMINATION

Weigh 100 g of the sample into an 800 cc beaker. Carefully decompose by adding, in small portions at a time and with stirring until the reaction ceases, 150 cc of the dilute HCl. Transfer the resulting mixture to a 600 cc beaker and dilute with $H_2O$ to a volume of 500 cc. Place the paddle of the stirring apparatus in the beaker and so adjust that the blades of the paddle will clear the sides and bottom of the beaker. Next insert well within the body of the liquid but above the blades of the stirring paddle the two electrodes, so arranged that they will be about 1 cm apart. This is conveniently done by passing the glass tubes thru the two holes of a No. 4 rubber stopper fastened to a ring stand by means of a clamp which will permit adjusting to any desired height above the beaker. Make the connections with the electric current and start the stirring apparatus. By means of the rheostat adjust the flow of the current to not more than 0.08 nor less than 0.05 amperes. Electrolize overnight at room temp. Upon completion of the electrolysis, without interrupting the current remove the electrodes from the soln, place in a beaker of fresh $H_2O$, and continue the flow of the electric current for 15 min. Remove the electrodes and break the electric current. Wash the cathode lightly with $H_2O$ from a wash bottle, place in a 50 cc beaker, and remove lead by adding 5 cc of the dilute $HNO_3$, warming on a steam bath, if necessary. Wash the cathode with a little more of the dilute $HNO_3$, receiving the washings in the beaker containing the dissolved Pb. Evaporate the contents of the beaker to dryness on a steam bath and take up the residue with 20 cc of the dilute acetic acid, warming, if necessary. If the soln is not clear, filter and wash the residue with the dilute acetic acid. Add 1 cc of a 1% soln of Na-acetate and 2 cc of the $K_2Cr_2O_7$ soln and allow to stand for 1 hour on a steam bath or overnight at room temp. Decant thru a weighed Gooch crucible with an asbestos mat previously heated at 140–150°; then wash the precipitate several times by decantation and finally on the filter with boiling $H_2O$ until the washings are colorless. Dry the crucible and contents at 140–150° to constant weight. From the weight of the $PbCrO_4$ calculate the weight of Pb, using the factor 0.641.

## *II. Gravimetric Method*[16]*—Tentative*

### 36 REAGENTS

(a) *Sulfuric acid.*—Mix 500 cc of $H_2SO_4$ with 2500 cc of $H_2O$, let stand overnight, and filter.

(b) *Acid-alcohol-water mixture.*—Mix 80 cc of $H_2SO_4$ with 3 liters of $H_2O$, add 800 cc of redistilled 95% alcohol, stir thoroly, let stand overnight, and filter.

(c) *Glacial acetic acid.*—Redistil glacial acetic acid and store in bottles made from Pb-free glass.

(d) *Alkaline ammonium acetate soln.*—Mix 350 cc of the redistilled glacial acetic acid with 650 cc of $H_2O$, dilute 500 cc of strong $NH_4OH$ with 500 cc of $H_2O$, and mix the two solns. Store in bottles made from Pb-free glass.

(e) *Potassium chromate soln.*—Dissolve 65 g of $K_2CrO_4$ in 100 cc of $H_2O$ by heating gently. Allow the soln to come to room temp. and filter.

### 37 DETERMINATION

Weigh 100 g of the sample, prepared as directed under **1**, transfer to a 2-liter, lipped beaker, and add in small portions at a time 750 cc of the dilute $H_2SO_4$. When frothing has ceased mark the volume of the mixture on the side of the beaker. Heat on a hot plate to boiling and continue boiling for 3–4 min.; then heat on a steam bath until the starch is hydrolized, which procedure requires 20–30 min. The mixture will have a yellow color. Remove from the steam bath and add with stirring $CaSO_4$ that has been finely powdered in a mortar and rubbed with $H_2O$ to a thin paste. It is not necessary to add $CaSO_4$ because monocalcium phosphate baking powder itself forms sufficient insoluble residue. To combination baking powders containing in part monocalcium phosphate add 10 g of $CaSO_4$, and to other baking powders add 15 g of $CaSO_4$. Cool, dilute to the original volume with $H_2O$, add with stirring 1 liter of filtered 95% alcohol, cover beaker, and let stand overnight. By means of a siphon that can be controlled by a pinch-cock transfer the clear supernatant liquid to a Büchner funnel containing 3 layers of a very retentive, close-textured filter paper, using suction. To the moist residue in the beaker add 100 cc of the acid-alcohol-water mixture, stir well, let settle, and decant thru the same filter. Repeat this operation, using a fresh 100 cc portion of the acid-alcohol-water mixture. Wash the beaker, the residue, and the filter with alcohol, 70% by volume, passing the washing thru the filter until the filtrate is nearly free from acid. Discard the filtrate and washings and wash the filter flask. Transfer the filter and contents to the original beaker and extract the $PbSO_4$ from the residue by treating with 100 cc portions of the alkaline $NH_4$-acetate soln, heating to boiling, and filtering thru a new filter in an ordinary funnel. Five extractions are necessary. Transfer the filtrate, which will measure about 500 cc, to a lipped beaker; neutralize with the redistilled glacial acetic acid, using litmus paper as indicator; and add 10 cc of the glacial acetic acid in excess. Heat nearly to boiling. add 25 cc of the $K_2CrO_4$ soln, cover, and let stand 48 hours at room temp., stirring occasionally. Filter thru a weighed Gooch crucible, wash well with cold $H_2O$, dry at 125° for at least 45 min., cool in a desiccator, and weigh. The weight of $PbCrO_4$ multiplied by the factor 0.641 gives the weight of Pb.

## FLUORIDES[17]—TENTATIVE

### 38 REAGENTS

(a) *Sulfuric acid.*—98.5%. Add sufficient fuming $H_2SO_4$ to ordinary $H_2SO_4$ to give a soln containing about 99% of $H_2SO_4$. Heat in a large beaker, preferably on a hot plate, for about 1 hour after it begins to fume strongly in order to remove free $SO_2$ and $SO_3$. If the final product contains more than 98.5% of $H_2SO_4$, dilute with the correct quantity of $H_2SO_4$, or if it contains less than 98%, add more fuming acid. In either case the acid as finally used should contain 98–98.5% of $H_2SO_4$ as determined by titration with standard alkali.

(b) *Silver sulfate.*—Dissolve 10 g of $(Ag)_2SO_4$ in 100 cc of 98.5% $H_2SO_4$ and heat until the soln fumes strongly in order to remove volatile impurities.

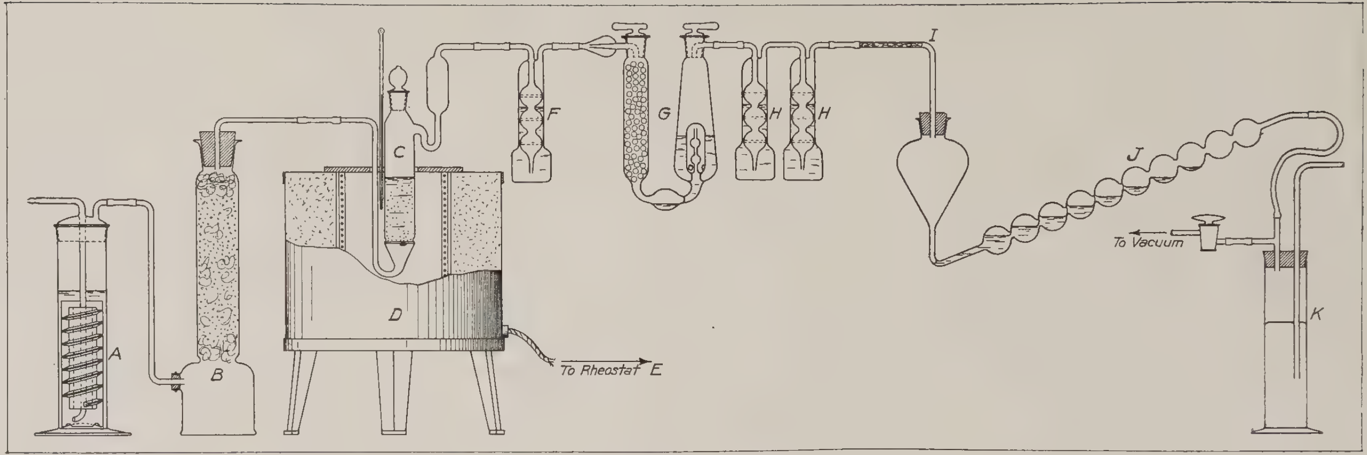
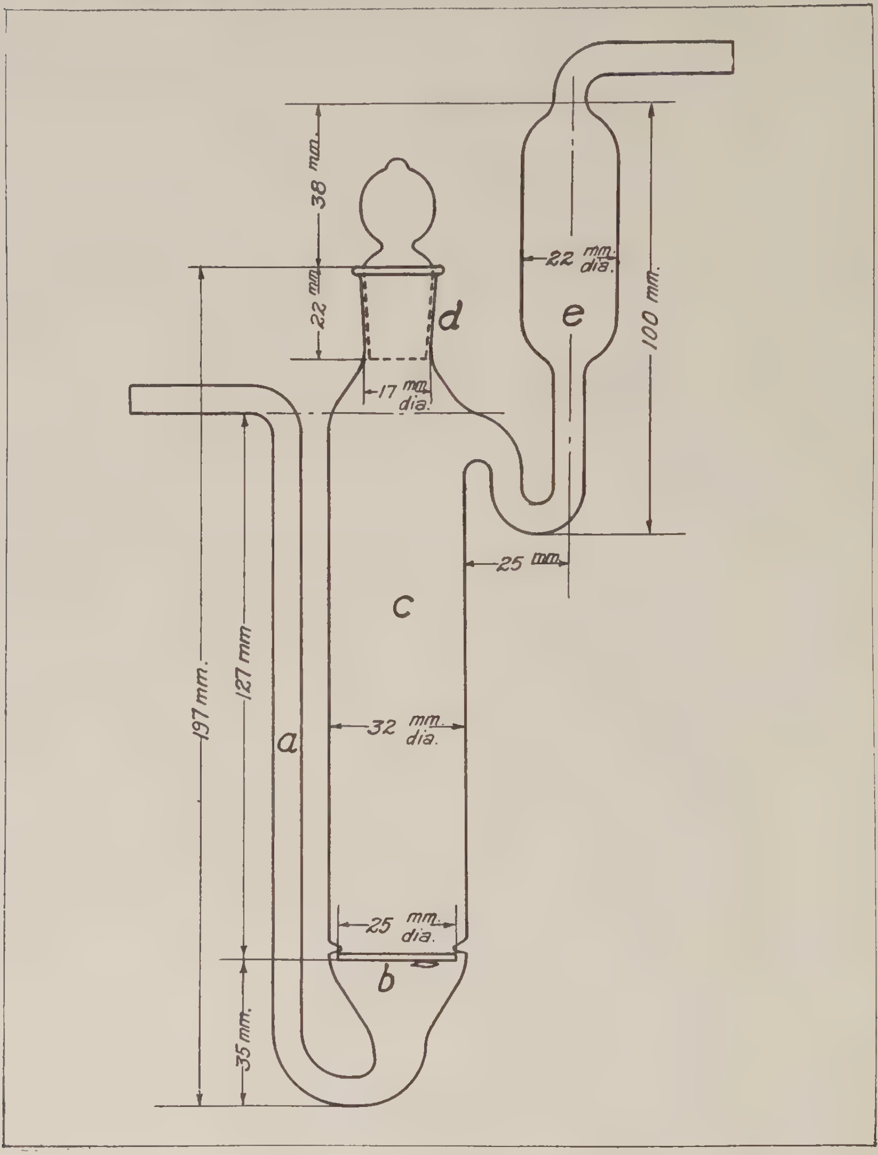

A
B
C
D
To Rheostat E
F
G
H
H
I
J
To Vacuum
K

FIG. 16.—APPARATUS FOR THE DETERMINATION OF FLUORINE

(c) *Chromium trioxide.*—Dry at 105–110°, grind to a fine powder, and make a suspension containing a large excess of the solid $CrO_3$ in 98.5% $H_2SO_4$.


38 mm.
22 mm.
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22 mm. dia.
e
100 mm.
17 mm dia.
25 mm.
c
197 mm.
127 mm
a
32 mm. dia.
25 mm. dia.
b
35 mm.

FIG. 17.—REACTION FLASK USED IN THE DETERMINATION OF FLUORINE

(d) *Silica.*—Grind the commercial grade of quartz flour to 200 mesh and ignite at a low red heat to remove moisture and organic matter.

(e) *Magnesium nitrate soln.*—Dissolve 10 g of $Mg(NO_3)_2$ in $H_2O$ and dilute to 100 cc.

(f) *Sodium hydroxide, hydrochloric acid and sulfuric acid solns.*—0.1 *N.*

### 39 APPARATUS

Fig. 16. (A) *Gas-washing bottle.*—Contains $H_2SO_4$ for removal of moisture from inlet air. (B) *Cylinder.*—Loosely packed with glass wool and $P_2O_5$, also for the removal of moisture. (C) *Reaction flask.*—For digesting the sample with 98.5% $H_2SO_4$. The flask, shown in detail in Fig. 17, is made of Pyrex glass and is designed especially for this purpose. It is composed of a principal tube (*c*), having an opening (*d*) for introducing the sample and acid, and it is closed with a ground-glass stopper, lubricated with 98.5% $H_2SO_4$. Dry air is drawn into the tube thru *a* and the gaseous reaction products pass out thru the bulbed tube *e*. The porcelain plate (*b*), about 2.5 cm in diameter and having perforations about 0.3 mm in diameter, makes close contact with the walls of the tube *c* and is held in place by indentations in the glass above and below the plate. (D) *Furnace for heating the reaction tube.*—A multiple unit, electric crucible furnace with rheostat is satisfactory and convenient for this purpose. (F) *Bowen potash tube.*—Contains 98.5% $H_2SO_4$. (G) *Schmidt $H_2SO_4$ tube.*—Contains a soln of $(AG)_2SO_4$ in 98.5% $H_2SO_4$. The cylindrical arm of the tube is filled with glass beads moistened with the $(Ag)_2SO_4$ soln. (H) 2 *Bowen potash tubes.*—The exit end of the first is fused to the inlet end of the second, and filled with the suspension of $CrO_3$ in 98.5% $H_2SO_4$. The contents of the tubes are renewed when most of the $CrO_3$ has been removed from suspension. (I) *Glass tube.*—Filled with glass wool. (J) *Meyer sulfur tube.*—Contains 50 cc of distilled $H_2O$ and 10 cc of 0.1 *N* HCl and is connected to the tube *I* by means of a rubber stopper. The exit end of the Meyer tube is connected with a source of vacuum thru a pressure regulating vessel (K) containing Hg. All connections are made with a good grade of rubber tubing and are coated with shellac. When not in use the apparatus should be closed to prevent the absorption of moisture from the air. It is imperative that all parts of the apparatus be perfectly dry and that water be absent from the reagents used.

### 40 PRELIMINARY TREATMENT

(a) Test the material for alkalinity by digesting about 5 g of the sample with 50 cc of $H_2O$, filtering, and adding a drop of phenolphthalein indicator to the filtrate. If acid, add sufficient CaO to the weighed portion taken for analysis to insure an alkaline reaction but avoid a large excess.

(b) Weigh 20 g of the sample, prepared as directed under (a), into an 11 cm porcelain evaporating dish; make alkaline as directed under (a), if necessary; place in a muffle furnace; and ignite at a temp. below discernible redness (about 450°). The resulting ash will be quite dark. Remove the dish from the muffle, allow to cool, powder the charred mass, and thoroly saturate it with 5 cc of $Mg(NO_3)_2$ soln. Remove excess moisture on a steam bath and reheat in the muffle furnace as before, repeating if necessary to destroy the C present. Keep in a desiccator until used.

### 41 DETERMINATION

Mix the ash obtained under **40** (b) with 1 g of 200-mesh $SiO_2$ and transfer to the dry digestion flask. Connect the flask in its place in the train and add 40 cc of 98.5% $H_2SO_4$. If the sample contains considerable carbonate, add the acid slowly from a dropping funnel connected with the digestion flask thru a rubber stopper while a slow current of air is drawn thru the apparatus. When all acid has been added quickly replace the funnel and rubber stopper by the ground-glass stopper, and draw air thru the apparatus at such a rate that a more or less continuous stream of bubbles is obtained. Raise the temp. of the digestion tube to about 300° in the course of about 45 min. Heat at this temp. for about 15 min. and then shake at intervals to facilitate soln of the white scum that forms on the surface of the acid. (The presence of Fl is characterized by the formation of this white scum.) Digest for about 1 hour, then discontinue heating, and continue aeration for 15–20 min. longer. Disconnect the Meyer sulfur-tube, transfer its contents to a 500 cc flask, and make up the final volume of soln and rinsings to 200–250 cc. Place 10 cc of the standard HCl in another flask of the same size and dilute to the same volume. Heat both flasks to boiling and boil for 5 min. Cool to about 50° and titrate the contents of each flask with 0.1 *N* NaOH, using phenolphthalein as indicator. To correct for sulfate that may be present, add a small quantity of 10% $BaCl_2$ to the titrated soln after acidifying with HCl and compare the resulting turbidity of the soln with that produced on adding 0.05–0.3 cc of 0.1 *N* $H_2SO_4$ to equal volumes of solns containing the same quantities of $BaCl_2$ and HCl.

From the total NaOH titration subtract the equivalents of the F blank on the reagents, of the HCl used in the absorption tube, and of the sulfate found in the titrated soln to obtain the net NaOH equivalent to the F content of the sample. 1 cc of 0.1 *N* NaOH soln = 0.0019 g of F.

### 42 ARSENIC—TENTATIVE

Introduce 5 g of the sample directly into the generator described under **XXIX, 2**; add 10 cc of $H_2O$, a little at a time to prevent foaming over, and then 15 cc of As-free HCl, introducing it drop by drop until foaming ceases. Heat on a steam bath until a drop of the mixture, when diluted and treated with I soln, shows no blue color. Then dilute to about 30 cc with $H_2O$ and continue from this point as directed under **XXIX, 4**, beginning with "Add 5 cc of KI reagent." Make the blank and the standards for comparison by the use of the As-free HCl of the same concentration as that used in the determination.

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[2] J. Am. Chem. Soc., **18**, 1 (1896).

[3] J. Assoc. Official Agr. Chem., **6**, 453 (1923).

[4] Ibid., **10**, 36 (1927).

[5] Conn. Agr. Exp. Sta. Rpt. 1900 (II), p. 169; Inland Revenue Dept. Canada. Bull., **68**, p. 31; Catlin, Baking Powders; A Treatise on Their Character, Methods for Determination of Their Values, etc., p. 20.

[6] J. Assoc. Official Agr. Chem., **6**, 454 (1923).

[7] Ibid., **8**, 91 (1924).

[8] Analyst, **47**, 339 (1922).

[9] Ann. chim. anal., **4**, 263 (1899).

[10] Conn. Agr. Exp. Sta. Rpt. 1900 (II), p. 174.

[11] J. Am. Chem. Soc., **47**, 142 (1925).

[12] J. Assoc. Official Agr. Chem., **12**, 46 (1929).

[13] Conn. Agr. Exp. Sta. Rpt. 1900 (II), p. 178.

[14] U. S. Dept. Agr. Bur. Chem. Bull. **13** (V), p. 596; Conn. Agr. Exp. Sta. Rpt. 1900 (II), p. 179.

[15] J. Assoc. Official Agr. Chem., **8**, 92 (1924).

[16] Ibid., **5**, 514 (1922).

[17] Ibid., **8**, 101 (1924); **11**, 225 (1928).

# XVI. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

### 1 SPECIFIC GRAVITY—OFFICIAL

Proceed as directed under **XVII, 24.**

### 2 ALCOHOL—OFFICIAL

Proceed as directed under **XVII, 25.**

### 3 TOTAL ACIDITY—OFFICIAL

Proceed as directed under **XXVI, 26.**

### 4 VOLATILE ACIDS—OFFICIAL

Proceed as directed under **XVII, 45** or **46.**

### 5 ESTERS—OFFICIAL

Proceed as directed under **XVII, 65.**

### 6 TOTAL SOLIDS—OFFICIAL

Proceed as directed under **XXXIV, 3** or **4.**

## SUCROSE

### 7 *By Polarization—Official*

Determine by polarizing before and after inversion as directed under **XXXIV, 22, 23** or **25.**

### 8 *By Reducing Sugars Before and After Inversion—Official*

Proceed as directed under **XXXIV, 28.**

### 9 REDUCING SUGARS—OFFICIAL

Proceed as directed under **XXXIV, 38,** and express the results as invert sugar.

### 10 COMMERCIAL GLUCOSE—OFFICIAL

Proceed as directed under **XXXIV, 30.**

### 11 CITRIC ACID—TENTATIVE

Proceed as directed under **XXVI, 33.**

### 12 TARTARIC ACID—TENTATIVE

Proceed as directed under **XXVI, 30.**

### 13 ASH—OFFICIAL

Proceed as directed under **XXXIV, 8** or **9.**

### 14 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 12.**

### 15 ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 13.**

### 16 ALKALINITY OF THE INSOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 14.**

### 17 ANALYSIS OF THE ASH—OFFICIAL

Proceed as directed under XII and XXVI.

### 18 PRESERVATIVES—OFFICIAL

Proceed as directed under XXXII.

### 19 COLORING MATTERS—TENTATIVE

Proceed as directed under XXI.

### 20 METALS—TENTATIVE

Proceed as directed under XXIX.

## ANTHRANILIC ACID ESTER

### *Colorimetric Method*[1]*—Official*

(Use when the sample contains less than 500 mg per liter.)

### 21 REAGENTS

(a) *Hydrochloric acid soln.*—Dilute 83 cc of HCl to 1 liter with $H_2O$.

(b) *Sodium nitrite soln.*—Dissolve 2 g of $NaNO_2$ in 100 cc of $H_2O$.

(c) *Hydrazine sulfate soln.*—Dissolve approximately 3 g of hydrazine sulfate in 100 cc of $H_2O$.

(d) *Sodium-α-naphthol-2-sulfonate soln.*—Dissolve 5 g of the sulfonate in 100 cc of $H_2O$.

(e) *Sodium carbonate soln.*—Dissolve 25 g of $Na_2CO_3$ in 75 cc of $H_2O$.

(f) *Standard soln of methyl anthranilate.*—Dissolve 0.25 g of methyl anthranilate in 60 cc of 95% (by volume) alcohol and dilute with $H_2O$ to 250 cc.

### 22 APPARATUS

(a) *Steam generator filled with $H_2O$.*—An oil can holding 1 gallon will serve the purpose.

(b) *Distillation flask.*—A Kjeldahl flask of about 750 cc capacity, with shortened neck, about 10 inches in height over all.

(c) *Spray tube.*—A glass tube with a small perforated bulb at the end. Passes thru a rubber stopper and reaches to the bottom of the distillation flask.

(d) *Connecting bulb.*—A Kjeldahl bulb with bent connecting tube.

(e) *Worm condenser.*—Having a water jacket 10–12 inches long. The outlet tube is extended to reach into the bottom of the receiving flash.

(f) *Receiving flask.*—A 500 cc Erlenmeyer flask.

### 23 DETERMINATION

Place enough $H_2O$ in the receiving flask to just cover or seal the end of the extended condenser tube. Place 10–100 cc of the sample of flavor in the distillation flask; add, if necessary, sufficient $H_2O$ to make the volume 100 cc; insert the stopper carrying the spray tube and connecting bulb; and connect with the condenser and receiving flask. Immerse the distillation flask in a water bath to the level of the contents, and when the sample has attained the temp. of the nearly boiling bath connect with the steam generator and pass a rapid current of steam thru the sample until about 300 cc of distillate has been collected.

Disconnect the apparatus and wash out the condenser with a little $H_2O$. Add to the distillate 25 cc of the HCl soln and 2 cc of the $NaNO_2$ soln, mix well, and let stand for exactly 2 min. Add 6 cc of the saturated soln of hydrazine sulfate and mix well for a min., so that the liquid comes in contact with all parts of the flask that may have been touched by the soln when it contained free nitrous acid. Keep the liquid in the flask in rapid motion, add quickly 5 cc of the Na-$\alpha$-naphthol-2-sulfonate soln, and then add immediately 15 cc of the $Na_2CO_3$ soln. Dilute the colored soln to 500 cc with $H_2O$, mix, and compare the color of an aliquot with the color of a standard or set of standards, prepared as nearly as possible at the same time. Calculate and express results as mg of anthranilic acid ester, as methyl anthranilate, per liter of sample.

### *Gravimetric Method*[2]*—Official*

(Use when the sample contains 500 mg or more per liter.)

### 24 REAGENTS

(a) *Hydrochloric acid soln.*—Dilute 83 cc of HCl to 1 liter with $H_2O$.

(b) *Sodium nitrite soln.*—Dissolve 2 g of $NaNO_2$ in 100 cc of $H_2O$.

(c) *$\alpha$-naphthol soln.*—Dissolve 0.2 g of $\alpha$-naphthol in 100 cc of 30% (by volume) alcohol.

(d) *Sodium bicarbonate soln.*—Dissolve 8.4 g of $NaHCO_3$ in 100 cc of $H_2O$.

### 25 APPARATUS

The apparatus used is described under **22**.

### 26 DETERMINATION

Place in the distillation flask a quantity of the sample of flavor that contains from 50–125 mg of anthranilic acid ester and dilute, if necessary, to 100 cc with $H_2O$. Subject the sample to steam distillation as directed in **23**, collecting about 400 cc of distillate. Have the $H_2O$ in the bath near the boiling point when the bath is placed under the distillation flask, also have the $H_2O$ in the steam generator boiling, and make the connection immediately.

Wash out the condenser with a little $H_2O$ and dilute the distillate to 500 cc. Mix, and to a 200 cc aliquot add 5 cc of the HCl soln and 5 cc of the $NaNO_2$ soln. Mix well and let stand for 1 min. Mix 25 cc of the $\alpha$-naphthol soln and 6 cc of the $NaHCO_3$ soln, pour the diazotized soln into the mixture, and let stand for 10 min. Fold two Whatman No. 1 or S. & S. No. 595 filter papers, 12.5 cm in diameter, and determine the difference in their weights by placing one on each pan of the balance and counterpoising with added weights. Place the heavier inside the lighter paper, fit into a funnel, and moisten. Pour the mixture thru this filter and wash the precipitate 7 or 8 times, using a total of about 100 cc of $H_2O$ for this purpose. Fill the filter only to within 1 cm of the top. Place the funnel carrying the filter and washed precipitate in an oven, and dry for about 10 min. at a temp. of 100°. Then separate the filter papers and dry them for about 1 hour at the same temp. Ascertain the difference in weights, dry again, weigh again, and repeat this procedure until the difference in weights remains constant. From this constant difference in weights subtract the original difference in weights of the 2 filter papers and multiply the result by 0.4935 to obtain the weight of anthranilic acid ester, as methyl anthranilate. Calculate and express as grams per liter of sample.

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[2] Ibid., 47.

# XVII. BEERS, WINES AND DISTILLED LIQUORS

(Unless otherwise noted express results as grams per 100 cc)

## BEERS

### 1 PREPARATION OF SAMPLE—OFFICIAL

Remove $CO_2$ by transferring the sample to a large flask and shaking vigorously or by pouring back and forth between beakers. The temp. of the beer should not fall below 20°.

### 2 COLOR—TENTATIVE

Determine the depth of color of the sample in a $\frac{1}{4}$ inch cell with a Lovibond tintometer, using the beer scale. Express the result in terms of a $\frac{1}{4}$ inch cell.

### 3 SPECIFIC GRAVITY*—OFFICIAL

Determine the specific gravity at 20/4° by means of a pycnometer, as directed under **24.**

### 4 ALCOHOL—OFFICIAL

Determine as directed under **25.**

### EXTRACT

### 5 *Method I—Official*

Measure, at 20°, 25 cc of the $CO_2$-free beer into a weighed, flat-bottomed Pt dish, approximately 85 mm in diameter; evaporate just to dryness on a steam bath; and heat to constant weight at 70° under a pressure of not to exceed 100 mm of Hg.

### 6 *Method II—Tentative*

The immersion refractometer reading of the beer at 20° − the immersion refractometer reading of the distillate at $20° \times 0.2571 =$ the grams of extract in 100 cc of beer.

### 7 *Method III—Official*

Calculate the specific gravity of the dealcoholized beer by the following formula:

$S = G + 1 - A$, in which
$S =$ the sp. gr. of the dealcoholized beer;
$G =$ the sp. gr. of the beer; and
$A =$ the sp. gr. of the distillate obtained in the determination of alcohol, **25.**

From Table 5 under **XLII**, ascertain the percentage by weight of extract in the dealcoholized beer corresponding to the value of $S$. Multiply the figure thus obtained by $S$ to obtain the grams of extract per 100 cc of beer.

### 8 EXTRACT OF ORIGINAL WORT (APPROXIMATE)—OFFICIAL

Calculate the grams of extract per 100 cc in the original wort by the following formula:

$O = 2A + E$, in which
$O =$ extract of the original wort;
$A =$ alcohol (g per 100 cc); and
$E =$ extract of the dealcoholized beer (g per 100 cc).

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* See note 9, p. xvii.

### 9 DEGREE OF FERMENTATION—OFFICIAL

Calculate the degree of fermentation by the following formula:

$$D=\frac{100\times 2A}{O}, \text{ in which}$$

$D$ = degree of fermentation;
$A$ = alcohol (g per 100 cc); and
$O$ = extract of original wort.

### 10 TOTAL ACIDS—OFFICIAL

Proceed as directed under **44.** Express the result as lactic acid, g per 100 cc; 1 cc of 0.1 $N$ NaOH = 0.0090 g of lactic acid.

### 11 VOLATILE ACIDS—OFFICIAL

Proceed as directed under **46.** Express the result as acetic acid, g per 100 cc.

### 12 REDUCING SUGARS—OFFICIAL

Dilute 25 cc of the $CO_2$-free beer, measured at 20°, with $H_2O$ at the same temp. to 100 cc. Determine the reducing sugars in 25 cc of this soln as directed under **XXXIV, 50.** Express the result as grams of anhydrous maltose per 100 cc of beer.

### 13 DEXTRIN—TENTATIVE

To 50 cc of the $CO_2$-free beer, measured at 20°, add 15 cc of HCl (sp. gr. 1.125); dilute to 200 cc; attach to a reflux condenser; and keep in a boiling water bath for 2 hours. Cool, nearly neutralize with NaOH soln, complete to a volume of 250 cc, filter, and determine dextrose as directed under **XXXIV, 59** or **61.** From the number of g of dextrose per 100 cc of beer, subtract 1.053 times the quantity of maltose, as found under **12,** and multiply the remainder by 0.9 to obtain the number of g of dextrin per 100 cc of beer.

### 14 DIRECT POLARIZATION—TENTATIVE

Read the polarization of the original sample in degrees Ventzke in a 200 mm tube at 20°. If the beer is turbid, clarify by shaking with alumina cream, filter, and correct the reading for dilution.

### 15 GLYCEROL—OFFICIAL

Proceed as directed under **27.**

### 16 ASH—OFFICIAL

Evaporate to dryness 25 cc of the $CO_2$-free sample, measured at 20°, and proceed as directed under **XXVII, 8.**

### 17 PHOSPHORIC ACID—OFFICIAL

To 25 cc of the $CO_2$-free beer, measured at 20°, add 20 cc of 2% Ca-acetate soln; evaporate to dryness; and ignite at low redness to a white ash. Add 10–15 cc of boiling $HNO_3$ (1+9) and determine $P_2O_5$ as directed under **II, 10.**

### 18 PROTEIN—OFFICIAL

Measure, at 20°, 25 cc of the $CO_2$-free beer into a Kjeldahl digestion flask; add a small quantity of tannin to prevent frothing; evaporate to dryness; determine N as directed under **II, 19, 22** or **24**; multiply the result by 6.25; and calculate the percentage of protein.

### 19 PRESERVATIVES—OFFICIAL

Proceed as directed under **XXXII.**

### 20 COLORING MATTERS—TENTATIVE

Proceed as directed under **XXI.**

### 21 METALS—TENTATIVE

Proceed as directed under **XXIX.**

## WINES

### 22 PHYSICAL EXAMINATION—TENTATIVE

Note and record the following: (1) Whether the container is "bottle full"; (2) the appearance of the wine, whether it is bright or turbid and whether there is any sediment; (3) condition when opened, whether still, gaseous, or carbonated; (4) color and depth of color; (5) odor, whether vinous, acetous, pleasant, or foreign; and (6) taste, whether vinous, acetous, sweet, dry, or foreign.

### 23 PREPARATION OF SAMPLE—OFFICIAL

If gas is contained in the wine, remove it by pouring the sample back and forth in beakers.

Filter the wine, regardless of appearance, before analyzing and determine immediately the specific gravity and such ingredients as alcohol, acids, and sugars, which are liable to change thru exposure.

### 24 SPECIFIC GRAVITY*—OFFICIAL

Determine the specific gravity at 20/4° (in vacuo) by means of a pycnometer as follows: Carefully clean the pycnometer by filling with a saturated soln of $CrO_3$ in $H_2SO_4$, allowing to stand for several hours, emptying, and rinsing thoroly with $H_2O$. Fill the pycnometer with recently boiled distilled $H_2O$ previously cooled to 16–18°, place in a water bath cooled to the same temp. and allow the bath to warm slowly to 20°. Adjust the level of the $H_2O$ to the proper point on the pycnometer, put the perforated cap or stopper in place, remove from the bath; wipe dry with a clean cloth, and after allowing to stand for 15–20 min., weigh. Empty, rinse several times with alcohol and then with ether, remove the ether fumes, allow the instrument to become perfectly dry, and weigh. Ascertain the weight of contained $H_2O$ at 20° in air ($W$ of the formula below) by subtracting the weight of the empty pycnometer from its weight when full. Cool the sample to 16–18°, adjust the level of the liquid to the proper point on the pycnometer, put the perforated cap or stopper in place, wipe dry, and weigh as before. Ascertain the weight of the contained sample at 20° in air ($S$ of the formula below) by subtracting the weight of the empty pycnometer from its weight when filled with the sample. Calculate the specific gravity in vacuo by the following formula:

$$G = \frac{S + 0.00105W}{1.00282W}, \text{ in which}$$

$G$ = corrected specific gravity of sample at 20/4° in vacuo;
$W$ = weight of contained $H_2O$ at 20° in air; and
$S$ = weight of contained sample at 20° in air.

### 25 ALCOHOL—OFFICIAL

(a) *By volume.*—Measure 100 cc of the liquid at 20° into a 300–500 cc distillation flask and add 50 cc of $H_2O$. Attach the flask to a vertical condenser by means of a bent tube, distil almost 100 cc, and make to a volume of 100 cc at 20°. (Foaming, which sometimes occurs especially with young wines, may be prevented by the addition of a small quantity of tannin.) To determine the alcohol in wines that have undergone acetous fermentation and contain an abnormal quantity of acetic acid, exactly neutralize the portion taken with NaOH soln before distillation. (This is unnecessary, however, for wines of normal taste and odor.) Determine the specific gravity of the distillate at 20°/4° as directed under **24** and obtain the corresponding percentage of alcohol by volume from Tables **19-21, XLII.**

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* See note 9, p. xvii.

(**b**) *Grams per 100 cc.*—From the specific gravity of the distillate, obtained under (**a**), ascertain the corresponding alcohol content in g per 100 cc from Tables **19-21, XLII.**

(**c**) *By weight.*—Divide the number of g in the 100 cc of distillate, as obtained in (**b**), by the weight of the sample as calculated from its specific gravity.

(**d**) *By immersion refractometer.*—Verify the percentages of alcohol, as determined under (**a**) and (**c**), by ascertaining the immersion refractometer reading of the distillate and obtaining the corresponding percentages of alcohol from Tables **19-21, XLII.**

## GLYCEROL IN DRY WINES

### 26 *Method I (By Direct Weighing)—Official*

Evaporate 100 cc of the wine in a porcelain dish on a water bath to a volume of about 10 cc. Treat the residue with about 5 g of fine sand and 4–5 cc of milk of lime (containing 15 g of CaO per 100 cc) for each g of extract present and evaporate almost to dryness. Treat the moist residue with 50 cc of alcohol, 90% by volume; remove the substance adhering to the sides of the dish with a spatula; and rub the whole mass to a paste. Heat the mixture on a water bath, with constant stirring, to incipient boiling and decant the liquid thru a filter into a small flask. Wash the residue repeatedly by decantation with 10 cc portions of hot 90% alcohol until the filtrate amounts to about 150 cc. Evaporate the filtrate to a sirupy consistency in a porcelain dish on a hot, but not boiling, water bath; transfer the residue to a small glass-stoppered, graduated cylinder with 20 cc of absolute alcohol; and add 3 portions of 10 cc each of anhydrous ether, shaking thoroly after each addition. Let stand until clear, pour off thru a filter, and wash the cylinder and filter with a mixture of 2 parts of absolute alcohol to 3 parts of anhydrous ether, also pouring the wash liquor thru the filter. Evaporate the filtrate to a sirupy consistency, dry for an hour at the temp. of boiling $H_2O$, weigh, ignite, and weigh again. The loss on ignition gives the weight of glycerol.

### 27 *Method II (By Oxidation with Dichromate)—Official*

Evaporate 100 cc of the wine in a porcelain dish on a water bath, the temp. of which is maintained at 85–90°, to a volume of 10 cc. Treat the residue with about 5 g of fine sand and 5 cc of milk of lime (containing 15 g of CaO per 100 cc). Proceed from this point as directed under **XXXIII, 63**, beginning with the clause "evaporate almost to dryness with frequent stirring," except to dilute the soln of glycerol after treatment with $(Ag_2)CO_3$ and Pb-acetate to a volume of 100 cc instead of 50 cc. Observe the precautions given concerning the temp. at which all evaporations are to be made.

## 28 GLYCEROL IN SWEET WINES—OFFICIAL

With wines in which the extract exceeds 5 g per 100 cc, heat 100 cc to boiling in a flask and treat with successive small portions of milk of lime until the wine becomes first darker and then lighter in color. Cool, add 200 cc of 95% alcohol, allow the precipitate to subside, filter, and wash with 95% alcohol. Treat the combined filtrate and washings as directed under **26** or **27**.

### 29 GLYCEROL-ALCOHOL RATIO—OFFICIAL

Express this ratio as $X:100$, in which $X$ is obtained by multiplying the percentage weight of glycerol by 100 and dividing the result by the percentage of alcohol by weight.

## EXTRACT

### 30 *I. From the Specific Gravity of the Dealcoholized Wine—Official*

Calculate the specific gravity of the dealcoholized wine by the following formula:

$S=G+1-A$, in which
$S$ = specific gravity of the dealcoholized wine;
$G$ = specific gravity of the wine, **24**; and
$A$ = specific gravity of the distillate obtained in the determination of alcohol, **25** (**a**).

From Table **5**, under **XLII**, ascertain the percentage by weight of extract in the dealcoholized wine corresponding to the value of $S$. Multiply the figure thus obtained by the value of $S$ to obtain the g of extract per 100 cc of wine.

### 31 *II. By Evaporation—Official*

(**a**) *In dry wines, having an extract content of less than 3 grams per 100 cc.*—Evaporate 50 cc of the sample on a water bath to a sirupy consistency in a 75 cc flat-bottomed Pt dish, approximately 85 mm in diameter. Heat the residue for 2–5 hours in a drying oven at the temp. of boiling $H_2O$, cool in a desiccator, and weigh as soon as the dish and contents reach room temp.

(**b**) *In sweet wines.*—If the extract content is between 3 and 6 g per 100 cc, treat 25 cc of the sample as directed under (**a**). If the extract exceeds 6 g per 100 cc, however, the result, obtained as directed under **30**, is accepted, and no gravimetric determination is attempted because of the inaccurate results obtained by drying levulose at a high temp.

### 32 NON-SUGAR SOLIDS—OFFICIAL

Determine the non-sugar solids (sugar-free extract) by subtracting the quantity of reducing sugars before inversion, **33**, from the extract, **30** or **31**. If sucrose is present in the wine, determine the non-sugar solids by subtracting the sum of reducing sugars before inversion and the sucrose from the extract.

### 33 REDUCING SUGARS—OFFICIAL

(**a**) *Dry wines.*—Place 200 cc of the wine in a porcelain dish; exactly neutralize with normal NaOH, calculating the quantity required from the determination of acidity, **44**; and evaporate to about $\frac{1}{4}$ the original volume. Transfer to a 200 cc flask, add sufficient neutral Pb-acetate soln to clarify, dilute to the mark with $H_2O$, shake, and pass thru a folded filter. Remove the Pb with dry K-oxalate and determine reducing sugars as directed under **XXXIV, 38.**

(**b**) *Sweet wines.*—With sweet wines, approximate the sugar content by subtracting 2 from the result in the determination of the extract and employ such a quantity of the sample that the aliquot taken for the Cu reduction shall not exceed 240 mg of invert sugar. Proceed as directed under (**a**) except to take this smaller quantity of the sample for the determination.

## SUCROSE

### 34 *I. By Reducing Sugars Before and After Inversion—Official*

Proceed as directed under **XXXIV, 28,** using the method given under **XXXIV, 38** for the determination of reducing sugars.

### 35 *II. By Polarization—Official*

Polarize before and after inversion in a 200 mm tube, as directed under **XXXIV, 22** or **23**, a portion of the filtrate obtained under **33**. In calculating the percentage of sucrose do not fail to take into consideration the relation of the weight of the sample contained in 100 cc to the normal weight for the instrument.

### 36 COMMERCIAL GLUCOSE—OFFICIAL

Polarize a portion of the filtrate obtained under **33**, after inversion in a 200 mm jacketed tube at 87°, as directed under **XXXIV, 29**. In calculating the percentage of glucose do not fail to take into consideration the relation of the weight of the sample contained in 100 cc to the normal weight for the instrument.

### 37 ASH—OFFICIAL

Proceed as directed under **XXVII, 8**, using the residue from 50 cc of the wine.

### 38 ASH-EXTRACT RATIO—OFFICIAL

Express results as $1:X$, in which $X$ is the quotient obtained by dividing the g of extract per 100 cc by the g of ash per 100 cc.

### 39 ALKALINITY OF THE WATER-SOLUBLE ASH—OFFICIAL

Extract the ash obtained as directed under **37** with successive small portions of hot $H_2O$ until the filtrate amounts to about 60 cc and proceed as directed under **XXXIV, 13**. Express the alkalinity in terms of the number of cc of 0.1 $N$ acid required to neutralize the water-soluble ash from 100 cc of the wine.

### 40 ALKALINITY OF THE WATER-INSOLUBLE ASH—OFFICIAL

Ignite the filter and residue from **39** in the Pt dish in which the wine was ashed and proceed as directed under **XXXIV, 14**. Express the alkalinity in terms of the number of cc of 0.1 $N$ acid required to neutralize the water-insoluble ash from 100 cc of the wine.

### 41 PHOSPHORIC ACID—OFFICIAL

Dissolve the ash obtained as directed under **37** in 50 cc of boiling $HNO_3$ (1+9), filter, wash the filter, and determine $P_2O_5$ in the combined filtrate and washings as directed under **II, 7** or **10**. If the ash ignites without difficulty, no free phosphoric acid need be suspected. Should there be any free acid, the ash remains black even after repeated leaching. In such cases, add Ca-acetate or a mixture containing 3 parts of $Na_2CO_3$ and 1 part of $NaNO_3$ to avoid loss of $P_2O_5$ before attempting to ash.

### 42 SULFURIC ACID—OFFICIAL

Precipitate directly the $H_2SO_4$ in 50 cc of the wine by means of 10% $BaCl_2$ soln after acidifying with a small excess of HCl, and determine the resulting $BaSO_4$ as directed under **XII, 25**. Allow the precipitate to stand for at least 6 hours before filtering. Report as $SO_3$, using the factor 0.3430.

### 43 CHLORIDES—OFFICIAL

To 100 cc of dry wine or 50 cc of sweet wine, add sufficient $Na_2CO_3$ to make distinctly alkaline. Evaporate to dryness, ignite at a heat not above low redness, cool, extract the residue with hot $H_2O$, acidify the water extract with $HNO_3$ (1+4), and determine chlorides as directed under **XII, 33** or **35**.

### 44 TOTAL ACIDS—OFFICIAL

Measure 20 cc of the wine into a 250 cc beaker, heat rapidly to incipient boiling, and immediately titrate with 0.1 *N* NaOH soln. Determine the end point with neutral 0.05% azolitmin soln as an outside indicator. Place the indicator in the cavities of a spot plate and spot the wine into the azolitmin soln. The end point is reached when the color of the indicator remains unchanged by the addition to the wine of a few drops of 0.1 *N* alkali.

In the case of wines that are artificially colored and therefore cannot be titrated satisfactorily in the above manner, it will be found helpful to use phenolphthalein powder (one part of phenolphthalein mixed with 100 parts of dry, powdered $K_2SO_4$) as an indicator. Place this indicator in the cavities of a spot plate and spot the wine into the powder. The end of the titration is indicated when the powder acquires a pink tint.

Express the result in terms of tartaric acid. 1 cc of 0.1 *N* NaOH soln = 0.0075 g of tartaric acid.

## VOLATILE ACIDS

### 45 *Method I—Official*

Heat rapidly to incipient boiling 50 cc of the wine in a 500 cc distillation flask and pass steam thru until 15 cc of the distillate requires only 2 drops of 0.1 *N* NaOH soln for neutralization. Boil the $H_2O$ used to generate the steam several minutes before connecting the steam generator with the distillation flask in order to expel $CO_2$. Titrate rapidly with 0.1 *N* NaOH soln, using phenolphthalein indicator. The color should remain about 10 seconds. Express the result as acetic acid. 1 cc of 0.1 *N* NaOH soln = 0.0060 g of acetic acid.

### 46 *Method II*[1]*—Official*

Introduce 10 cc of the wine, previously freed from $CO_2$, into the inner tube of a modified Sellier distillation apparatus (Fig. 18); add a small piece of paraffin to prevent foaming; and adjust the tube and its contents in place within the larger flask, which contains 100 cc of recently boiled $H_2O$. Connect with a condenser as illustrated in the figure and distil by heating the outer flask. When 50 cc of the distillate has been collected, empty the receiver into a beaker and titrate with 0.1 *N* NaOH soln, using phenolphthalein indicator. Continue the distillation and titrate each

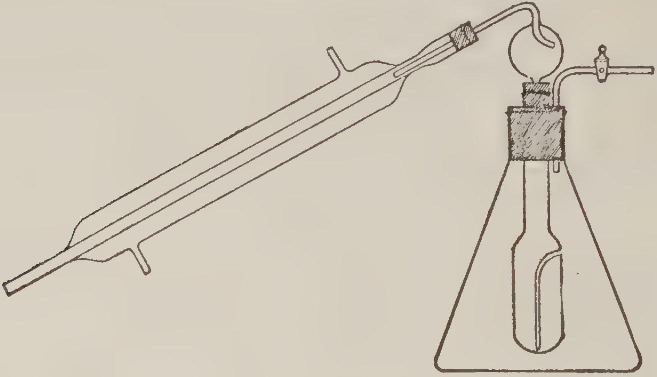

FIG. 18.—APPARATUS FOR THE DETERMINATION OF VOLATILE ACIDS

succeeding 10 cc of distillate until not more than 1 drop of standard alkali is required to reach the neutral point. Usually 80 cc of distillate will contain all the volatile acids.

### 47 FIXED ACIDS—OFFICIAL

To obtain the quantity of fixed acids, expressed as tartaric acid, multiply the quantity of volatile acids by 1.25 and subtract this product from the total acids.

### 48 TOTAL TARTARIC ACID[2]—OFFICIAL

Neutralize 100 cc of the wine with $N$ NaOH soln, calculating from the acidity, **44**, the number of cc of $N$ alkali necessary for the neutralization. If the volume of the soln is increased more than 10% by the addition of the alkali, evaporate to approximately 100 cc. Add to the neutralized soln 0.075 g of tartaric acid for each cc of $N$ alkali added and after the tartaric acid has dissolved add 2 cc of glacial acetic acid and 15 g of KCl. After the KCl has dissolved, add 15 cc of 95% alcohol; stir vigorously until the K-bitartrate begins to precipitate; and let stand in an ice box at 15–18° for at least 15 hours. Decant the liquid from the separated K-bitartrate on a Gooch crucible prepared with a very thin film of asbestos, or on filter paper in a Büchner funnel. Wash the precipitate and filter 3 times with a few cc of a mixture of 15 g of KCl, 20 cc of 95% alcohol, and 100 cc of $H_2O$, using not more than 20 cc of the wash soln in all. Transfer the asbestos or paper and precipitate to the beaker in which the precipitation was made; wash the Gooch crucible or Büchner funnel with hot $H_2O$, using about 50 cc in all; heat to boiling; and titrate the hot soln with 0.1 $N$ NaOH soln, using phenolphthalein indicator. Increase the number of cc of 0.1 $N$ alkali required by 1.5 cc to allow for the solubility of the precipitate. 1 cc of 0.1 $N$ alkali is equivalent, under these conditions, to 0.015 g of tartaric acid. To obtain the g of total tartaric acid per 100 cc of the wine, subtract the quantity of tartaric acid added from this result.

### 49 FREE TARTARIC ACID AND CREAM OF TARTAR[3]—OFFICIAL

Calculate the free tartaric acid and cream of tartar in the following manner:

Let $A$ = total tartaric acid in 100 cc of wine, divided by 0.015;
$B$ = total alkalinity of the ash (sum of $C$ and $D$);
$C$ = alkalinity of water-soluble ash; and
$D$ = alkalinity of water-insoluble ash.

Then

(1) If $A$ is greater than $B$,
Cream of tartar $= 0.0188 \times C$, and
Free tartaric acid $= 0.015 \times (A - B)$;

(2) If $A$ equals $B$ or is smaller than $B$ but greater than $C$
Cream of tartar $= 0.0188 \times C$, and
Free tartaric acid $= 0$; and

(3) If $A$ is smaller than $C$,
Cream of tartar $= 0.0188 \times A$, and
Free tartaric acid $= 0$.

## TANNIN AND COLORING MATTER—OFFICIAL

### 50 REAGENTS

(a) *Oxalic acid.*—0.1 $N$. 1 cc = 0.00416 g of tannin.

(b) *Standard potassium permanganate soln.*—Dissolve 1.333 g of $KMnO_4$ in 1 liter of $H_2O$ and standardize the soln against (a).

(c) *Indigo soln.*—Dissolve 6 g of Na-sulfindigotate in 500 cc of $H_2O$ by heating, cool, add 50 cc of $H_2SO_4$, make up to 1 liter, and filter.

(d) *Purified boneblack.*—Boil 100 g of finely powdered boneblack with successive portions of HCl (1+3), filter, and wash with boiling $H_2O$ until free from chlorides. Keep covered with $H_2O$.

### 51 DETERMINATION[4]

Dealcoholize 100 cc of the wine by evaporation and dilute with $H_2O$ to the original volume. Transfer 10 cc to a 2-liter porcelain dish and add about 1 liter of $H_2O$ and exactly 20 cc of the indigo soln. Add the standard $KMnO_4$ soln, 1 cc at a time, until the blue color changes to green; then add a few drops at a time until the color becomes golden yellow. Designate the number of cc of $KMnO_4$ soln used as "a."

Treat 10 cc of the dealcoholized wine, prepared as above, for 15 min. with boneblack; filter; and wash thoroly with $H_2O$. Add 1 liter of $H_2O$ and 20 cc of the indigo soln and titrate with $KMnO_4$, as above. Designate the number of cc of $KMnO_4$ used as "b."

Then $a-b=c$, the number of cc of the $KMnO_4$ soln required for the oxidation of the tannin and coloring matter in 10 cc of the wine.

### 52 CRUDE PROTEIN—OFFICIAL

Determine N in 50 cc of the wine as directed under **II, 19, 22** or **24,** and multiply the result by 6.25.

### 53 PENTOSANS—OFFICIAL

Proceed as directed under **XXVII, 29,** except to use 100 cc of the wine and 43 cc of HCl in beginning the distillation. Owing to the interference of sugars this determination can be made in dry wines only.

### 54 GUM AND DEXTRIN—TENTATIVE

Evaporate 100 cc of the wine to about 10 cc and add 10 cc of 95% alcohol. If gum or dextrin is present (indicated by the formation of a voluminous precipitate), continue the addition of alcohol, slowly and with stirring, until 100 cc has been added. Let stand overnight; filter; and wash with alcohol, 80% by volume. Dissolve the precipitate on the paper with hot $H_2O$, hydrolyze the filtrate and washings with HCl, and proceed as directed under **XXVII, 23.**

### 55 NITRATES—TENTATIVE

(a) *White wine.*—Treat a few drops of the wine in a porcelain dish with 2–3 cc of $H_2SO_4$ that contains about 0.1 g of diphenylamine[5] per 100 cc. The deep blue color formed in the presence of nitrates appears so quickly that it is not obscured, even in sweet wine, by the blackening produced by the action of $H_2SO_4$ on the sugar.

(b) *Red wine.*—Clarify with basic lead acetate, filter, remove the excess of Pb from the filtrate with $Na_2SO_4$, filter again, and treat a few drops of this filtrate as directed under (a).

### 56 COLORING MATTERS—TENTATIVE

Proceed as directed under **XXI.**

### 57 PRESERVATIVES—OFFICIAL

Proceed as directed under **XXXII.**

The detection of added boric acid is somewhat difficult because a small quantity of it is normally present in certain wines. Therefore a quantitative determination should be made. The determination of $SO_2$ must also be quantitative. A small quantity of salicylic acid is also normal in wine, and for that reason not more than 50 cc of the sample should be used in testing for that preservative.

## DISTILLED LIQUORS

**58**

### SPECIFIC GRAVITY*—OFFICIAL

Determine the specific gravity at 20/4° by means of a pycnometer, as directed under **24**, or by means of a small, accurately graduated hydrometer.

**59**

### ALCOHOL BY WEIGHT—OFFICIAL

Weigh 20–25 g of the sample into a distillation flask, dilute with 100 cc of $H_2O$, and distil nearly 100 cc. Weigh the distillate or make to volume at 20°. In either case determine the specific gravity as directed under **58**. Obtain the corresponding percentage of alcohol by weight from Tables **19-21, XLII**; multiply this figure by the weight of the distillate; and divide by the weight of the sample taken to obtain the percentage of alcohol by weight.

The alcohol content of the distillate may be checked by determining the immersion refractometer reading and obtaining the percentage of alcohol from Table **22** under **XLII.**

### ALCOHOL BY VOLUME

**60**

#### *Method I—Official*

From the specific gravity of the distillate obtained under **59** ascertain the corresponding percentage of alcohol by volume from Tables **19-21, XLII.** Multiply this figure by the volume of distillate and divide by the volume of the sample (calculated from the specific gravity) to obtain the percentage of alcohol by volume in the original sample.

**61**

#### *Method II—Official*

Measure 25 cc of the sample at 20° into a distillation flask, dilute with 100 cc of $H_2O$, distil nearly 100 cc, make to volume at 20°, and determine the specific gravity as directed under **24.** Obtain, from Tables **19-21, XLII,** the corresponding percentage of alcohol by volume in the distillate and multiply by 4 to obtain the percentage of alcohol by volume in the original substance.

The alcohol content of the distillate may be checked by determining the immersion refractometer reading and obtaining the percentage of alcohol from Tables **19-21, XLII.**

**62**

### EXTRACT—OFFICIAL

Weigh, or measure at 20°, 100 cc of the sample, evaporate nearly to dryness on a steam bath, transfer to a water oven, and dry at the temp. of boiling $H_2O$ for 2.5 hours.

**63**

### ASH—OFFICIAL

Proceed as directed under **XXVII, 8,** using the residue from the determination of the extract, **62.**

**64**

### ACIDITY—OFFICIAL

Titrate 100 cc of the sample (or 50 cc diluted to 100 cc if the sample is dark) with 0.1 $N$ alkali, using phenolphthalein indicator. Express the result as acetic acid. 1 cc of 0.1 $N$ alkali = 0.0060 g of acetic acid.

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* See note 9, p. xvii.

### 65 ESTERS—OFFICIAL*

Measure 100–200 cc of the sample into a distillation flask; add 12.5–25 cc of $H_2O$; and distil slowly 100–200 cc, depending upon the amount of sample taken, using a mercury valve to prevent loss of alcohol. Exactly neutralize the free acid in 50 cc of the distillate with 0.1 *N* alkali and add a measured excess of 25–50 cc of 0.1 *N* alkali. Then either boil for an hour under a reflux condenser, cool, and titrate with 0.1 *N* acid, or allow the soln to stand overnight in a stoppered flask with the excess of alkali, heat with a tube condenser for 30 min. at a temp. below the boiling point, cool, and titrate. Calculate the number of cc of 0.1 *N* alkali used in the saponification of the esters as ethyl acetate. 1 cc of 0.1 *N* alkali = 0.0088 g of ethyl acetate. Run a blank, using water in place of the distillate, and make any necessary correction.

## ALDEHYDES—OFFICIAL

### 66 REAGENTS

(a) *Aldehyde-free alcohol.*—Redistil 95% alcohol over NaOH or KOH; add 2–3 g per liter of meta-phenylenediamine hydrochloride; digest at ordinary temp. for several days (or under a reflux condenser on a steam bath for several hours); and distil slowly, rejecting the first 100 cc and the last 200 cc of the distillate.

(b) *Sulfite-fuchsin soln.*—Dissolve 0.50 g of pure fuchsin in 500 cc of $H_2O$, add 5 g of $SO_2$ dissolved in $H_2O$, make up to 1 liter, and allow to stand until colorless. As this soln decomposes rapidly, prepare it in small quantities and keep at a low temp.

(c) *Standard acetaldehyde soln.*—Prepare according to the directions of Vasey,[6] as follows: Grind aldehyde ammonia in a mortar with anhydrous ether and decant the ether. Repeat this operation several times and dry the purified salt in a current of air and then in vacuo over $H_2SO_4$. Dissolve 1.386 g of this purified aldehyde ammonia in 50 cc of 95% alcohol, add 22.7 cc of *N* alcoholic $H_2SO_4$, make up to 100 cc and add 0.8 cc of alcohol for the volume of the $(NH_4)_2SO_4$ precipitate. Allow the mixture to stand overnight, and filter. This soln contains 1 g of acetaldehyde in 100 cc and will retain its strength.

The standard found most convenient for use is 2 cc of this strong aldehyde soln diluted to 100 cc with alcohol, 50% by volume. 1 cc of this soln = 0.0002 g of acetaldehyde. Make up the soln every day or so, as it loses strength.

### 67 DETERMINATION

Determine the aldehyde in the distillate prepared as directed under **65**. Dilute 5–10 cc of the distillate to 50 cc with aldehyde-free alcohol, 50% by volume; add 25 cc of the sulfite-fuchsin soln; and allow to stand for 15 min. at 15°. The solns and reagents should be at 15° when they are mixed. Prepare standards of known strength and blanks in the same way. The comparison standards found most convenient for use contain 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg of acetaldehyde.

## FURFURAL—OFFICIAL

### 68 REAGENT

*Standard furfural soln.*—Dissolve 1 g of redistilled furfural in 100 cc of 95% alcohol. Prepare standards by diluting 1 cc of this soln to 100 cc with alcohol, 50% by volume. 1 cc of this soln contains 0.1 mg of furfural. (The strong furfural soln will retain its strength, but the dilute soln will not.)

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* The use of "100–200 cc" instead of "200 cc" and of "12.5–25 cc" instead of "25 cc" has been approved as official, first action.

### 69 DETERMINATION

Dilute 10–20 cc of the distillate, as prepared under **65**, to 50 cc with furfural-free alcohol, 50% by volume. Add 2 cc of colorless aniline and 0.5 cc of HCl (sp. gr. 1.125) and keep for 15 min. in a water bath at about 15°. Prepare standards of known strength and blanks in the same way. The comparison standards found most convenient for use contain 0.05, 0.1, 0.15; 0.2, 0.25, and 0.3 mg of furfural.

## FUSEL OIL—OFFICIAL*

### 70 REAGENTS

(a) *Purified carbon tetrachloride.*—Mix in a separatory funnel crude $CCl_4$ with $^1/_{10}$ its volume of $H_2SO_4$, shake thoroly at frequent intervals, and allow to stand overnight. Wash free of acid and impurities with tap $H_2O$, remove the $H_2O$, add an excess of NaOH soln, and distil the $CCl_4$

The refuse $CCl_4$ after titration is purified for further work by collecting in a large bottle, adding NaOH soln (1+1), shaking, washing with tap $H_2O$ until the washings are neutral to phenolphthalein, and distilling.

(b) *Oxidizing soln.*—Dissolve 100 g of $K_2Cr_2O_7$ in 900 cc of $H_2O$ and add 100 cc of $H_2SO_4$.

### 71 DETERMINATION

(1) To 50 cc of the sample add 50 cc of $H_2O$, then add 20 cc of 0.5 *N* NaOH, and saponify the mixture by boiling for an hour under a reflux condenser; or, (2) mix 50 cc of the liquid and 50 cc of $H_2O$ with 20 cc of 0.5 *N* NaOH, allow to stand overnight at room temp., and distil directly. Connect the flask with a distillation apparatus, distil 90 cc, add 25 cc of $H_2O$, and continue the distillation until an additional 25 cc is collected.

Whenever aldehydes are present in excess of 15 parts per 100,000, add to the distillate 0.5 g of metaphenylenediamine hydrochloride, boil under a reflux condenser for an hour, distil 100 cc, add 25 cc of $H_2O$, and continue the distillation until an additional 25 cc is collected.

Approximately saturate the distillate with finely ground NaCl and add saturated NaCl soln until the specific gravity is 1.10.

Extract this salt soln 4 times with the purified $CCl_4$, using 40, 30, 20, and 10 cc, respectively, and wash the $CCl_4$ 3 times with 50 cc portions of saturated NaCl soln, and twice with saturated $Na_2SO_4$ soln. Transfer the $CCl_4$ to a flask containing 50 cc of the oxidizing soln and boil for 8 hours under a reflux condenser.

Add 100 cc of $H_2O$ and distil until only about 50 cc remains. Add 50 cc of $H_2O$ and again distil until 35–50 cc is left. Use extreme care to prevent the oxidizing mixture from burning and baking on the side of the distilling flask. The distillate should be water white: if it is colored discard it and repeat the determination. Titrate the distillate with 0.1 *N* NaOH, using phenolphthalein indicator. 1 cc of 0.1 *N* NaOH = 0.0088 g of amyl alcohol.

If preferred, use rubber stoppers in the saponification and first distillation, but use corks covered with tinfoil in the oxidation and second distillation. Renew the corks and tinfoil frequently.

Conduct a blank determination upon 100 cc of $CCl_4$, beginning the blank at that point of the procedure immediately after the extraction and just before the washings with NaCl and $Na_2SO_4$ solns.

### 72 SUGARS—OFFICIAL

Proceed as directed under **34** or **35**.

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* In **71**, lines 1 and 3, the use of "50 cc" instead of "100 cc" has been approved as official, first action.

## METHYL ALCOHOL

### 73 *Trillat Method[7]—Official*

To 50 cc of the sample add 50 cc of $H_2O$ and 8 g of lime and fractionate by the aid of Glinsky bulb tubes. Dilute the first 15 cc of the distillate to 150 cc, mix with 15 g of $K_2Cr_2O_7$ and 70 cc of $H_2SO_4$ (1+5), and allow to stand for 1 hour, shaking occasionally.

Distil, reject the first 25 cc, and collect 100 cc. Mix 50 cc of the distillate with 1 cc of redistilled dimethylaniline, transfer to a stout tightly stoppered flask, and keep on a bath at 70–80° for 3 hours, shaking occasionally. Make distinctly alkaline with NaOH soln and distil off the excess of dimethylaniline, stopping the distillation when 25 cc has passed over.

Acidify the residue in the flask with acetic acid, shake, and test a few cc by adding 4 or 5 drops of a 1% suspension of $PbO_2$. If methyl alcohol is present, there occurs a blue coloration, which is increased by boiling. Ethyl alcohol thus treated yields a blue coloration which changes immediately to green, later to yellow, and becomes colorless when boiled.

### 74 *Riche and Bardy Method[8]—Official*

The following method depends on the formation of methylaniline violet:

Place 10 cc of the sample, previously redistilled over $K_2CO_3$ if necessary, in a small flask with 15 g of I and 2 g of red P. Keep in ice $H_2O$ for 10–15 min. or until action has ceased. Distil on a water bath into about 30 cc of $H_2O$, the methyl and ethyl iodides formed. Wash with dilute alkali to eliminate free I. Separate the heavy, oily liquid that settles and transfer to a flask containing 5 cc of aniline. If the action is too violent, place the flask in cold $H_2O$; if too slow, stimulate by gently warming the flask. After an hour boil the product with $H_2O$, cool, and add about 20 cc of 15% NaOH soln; when the bases rise to the top as an oily layer, fill the flask up to the neck with $H_2O$ and draw them off with a pipet. Oxidize 1 cc of the oily liquid by adding 10 g of a mixture of 100 parts of clean sand, 2 of NaCl, and 3 of $Cu(NO_3)_2$; mix thoroly; transfer to a glass tube; and heat to 90° for 8–10 hours. Exhaust the product with warm alcohol, filter, and dilute to 100 cc with alcohol. If the sample is free from methyl alcohol, the liquid has a red tint, but in the presence of 1% of methyl alcohol it has a distinct violet shade; with 2.5% the shade is very distinct and still more so with 5%. To detect more minute quantities of methyl alcohol, dilute 5 cc of the colored liquid to 100 cc with $H_2O$ and dilute 5 cc of this again to 400 cc. Heat the liquid thus obtained in a porcelain dish and immerse in it a fragment of white merino (free from S) for 30 min. If the alcohol is pure, the wool will remain white, but if methyl alcohol is present the fiber will become violet, the depth of tint giving a fairly approximate indication of the proportion of methyl alcohol.

### 75 *Immersion Refractometer Method[9]—Official*

Determine by the immersion refractometer at 20° the refraction of the distillate obtained in the determination of alcohol. If, on reference to the table under **76**, the refraction shows the percentage of alcohol agreeing with that obtained from the specific gravity, it may be assumed that no methyl alcohol is present. If, however, there is an appreciable quantity of methyl alcohol, the low refractometer reading will at once indicate the fact. If the absence from the soln of refractive substances other than $H_2O$ and the alcohols is assured, this difference in refraction is conclusive of the presence of methyl alcohol.

The addition of methyl alcohol to ethyl alcohol decreases the refraction in direct proportion to the quantity present; hence the quantitative calculation is made readily by interpolation in the table under **76** of the figures for pure ethyl and methyl alcohol of the same alcoholic strength as the sample being used.

*Example.*—The distillate has a specific gravity of 0.97080, corresponding to 18.38% alcohol by weight, and has a refraction of 35.8 at 20° by the immersion refractometer; by interpolation in the refractometer table the readings of ethyl and methyl alcohol corresponding to 18.38% alcohol are 47.3 and 25.4, respectively, the difference being 21.9; $47.3-35.8=11.5$; $(11.5 \div 21.9)100=52.5$, showing that 52.5% of the total alcohol present is methyl alcohol.

### 76

*Scale readings on Zeiss immersion refractometer at 20°, corresponding to each per cent by weight of methyl and ethyl alcohols.*

| PER CENT ALCOHOL BY WEIGHT | SCALE READINGS | | PER CENT ALCOHOL BY WEIGHT | SCALE READINGS | | PER CENT ALCOHOL BY WEIGHT | SCALE READINGS | | PER CENT ALCOHOL BY WEIGHT | SCALE READINGS | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Methyl alcohol | Ethyl alcohol | | Methyl alcohol | Ethyl alcohol | | Methyl alcohol | Ethyl alcohol | | Methyl alcohol | Ethyl alcohol |
| 0 | 14.5 | 14.5 | 25 | 29.7 | 60.1 | 50 | 39.8 | 90.3 | 75 | 29.7 | 101.0 |
| 1 | 14.8 | 16.0 | 26 | 30.3 | 61.9 | 51 | 39.7 | 91.1 | 76 | 29.0 | 101.0 |
| 2 | 15.4 | 17.6 | 27 | 30.9 | 63.7 | 52 | 39.6 | 91.8 | 77 | 28.3 | 100.9 |
| 3 | 16.0 | 19.1 | 28 | 31.6 | 65.5 | 53 | 39.6 | 92.4 | 78 | 27.6 | 100.9 |
| 4 | 16.6 | 20.7 | 29 | 32.2 | 67.2 | 54 | 39.5 | 93.0 | 79 | 26.8 | 100.8 |
| 5 | 17.2 | 22.3 | 30 | 32.8 | 69.0 | 55 | 39.4 | 93.6 | 80 | 26.0 | 100.7 |
| 6 | 17.8 | 24.1 | 31 | 33.5 | 70.4 | 56 | 39.2 | 94.1 | 81 | 25.1 | 100.6 |
| 7 | 18.4 | 25.9 | 32 | 34.1 | 71.7 | 57 | 39.0 | 94.7 | 82 | 24.3 | 100.5 |
| 8 | 19.0 | 27.8 | 33 | 34.7 | 73.1 | 58 | 38.6 | 95.2 | 83 | 23.6 | 100.4 |
| 9 | 19.6 | 29.6 | 34 | 35.2 | 74.4 | 59 | 38.3 | 95.7 | 84 | 22.8 | 100.3 |
| 10 | 20.2 | 31.4 | 35 | 35.8 | 75.8 | 60 | 37.9 | 96.2 | 85 | 21.8 | 100.1 |
| 11 | 20.8 | 33.2 | 36 | 36.3 | 76.9 | 61 | 37.5 | 96.7 | 86 | 20.8 | 99.8 |
| 12 | 21.4 | 35.0 | 37 | 36.8 | 78.0 | 62 | 37.0 | 97.1 | 87 | 19.7 | 99.5 |
| 13 | 22.0 | 36.9 | 38 | 37.3 | 79.1 | 63 | 36.5 | 97.5 | 88 | 18.6 | 99.2 |
| 14 | 22.6 | 38.7 | 39 | 37.7 | 80.2 | 64 | 36.0 | 98.0 | 89 | 17.3 | 98.9 |
| 15 | 23.2 | 40.5 | 40 | 38.1 | 81.3 | 65 | 35.5 | 98.3 | 90 | 16.1 | 98.6 |
| 16 | 23.9 | 42.5 | 41 | 38.4 | 82.3 | 66 | 35.0 | 98.7 | 91 | 14.9 | 98.3 |
| 17 | 24.5 | 44.5 | 42 | 38.8 | 83.3 | 67 | 34.5 | 99.1 | 92 | 13.7 | 97.8 |
| 18 | 25.2 | 46.5 | 43 | 39.2 | 84.2 | 68 | 34.0 | 99.4 | 93 | 12.4 | 97.2 |
| 19 | 25.8 | 48.5 | 44 | 39.3 | 85.2 | 69 | 33.5 | 99.7 | 94 | 11.0 | 96.4 |
| 20 | 26.5 | 50.5 | 45 | 39.4 | 86.2 | 70 | 33.0 | 100.0 | 95 | 9.6 | 95.7 |
| 21 | 27.1 | 52.4 | 46 | 39.5 | 87.0 | 71 | 32.3 | 100.2 | 96 | 8.2 | 94.9 |
| 22 | 27.8 | 54.3 | 47 | 39.6 | 87.8 | 72 | 31.7 | 100.4 | 97 | 6.7 | 94.0 |
| 23 | 28.4 | 56.3 | 48 | 39.7 | 88.7 | 73 | 31.1 | 100.6 | 98 | 5.1 | 93.0 |
| 24 | 29.1 | 58.2 | 49 | 39.8 | 89.5 | 74 | 30.4 | 100.8 | 99 | 3.5 | 92.0 |
| | | | | | | | | | 100 | 2.0 | 91.0 |

### 77 COLORING MATTERS—TENTATIVE

Proceed as directed under **XXI**.

### 78 WATER-INSOLUBLE COLOR—TENTATIVE

Evaporate 50 cc of the sample just to dryness on a steam bath. Take up with approximately 15 cc of cold $H_2O$, filter, and wash until the filtrate amounts to nearly 25 cc. To this filtrate add 25 cc of absolute alcohol, or 26.3 cc of 95% alcohol, and make up to 50 cc with $H_2O$. Mix thoroly and compare in a colorimeter with the original material. Calculate from these readings the percentage of color insoluble in $H_2O$.

### 79 COLORS INSOLUBLE IN AMYL ALCOHOL—TENTATIVE

Evaporate 50 cc of the sample just to dryness on a steam bath. Dissolve the residue in $H_2O$ and 95% alcohol and make to a volume of 50 cc, using a total volume of 26.3 cc of 95% alcohol. Place 25 cc of this soln in a separatory funnel and add 20 cc of freshly shaken Marsh reagent (100 cc of pure amyl alcohol, 3 cc of sirupy $H_3PO_4$, and 3 cc of $H_2O$), shaking lightly so as not to form an emulsion. Allow the layers to separate and repeat this shaking and standing twice. After the layers have separated completely draw off the lower or aqueous layer, which contains the caramel, into a 25 cc cylinder and make up to volume with alcohol, 50% by volume. Compare this soln in a colorimeter with the untreated 25 cc. Calculate from this reading the percentage of color insoluble in amyl alcohol.

### 80 CARAMEL[10]—TENTATIVE

Add 10 cc of paraldehyde to 5 cc of the sample in a test tube and shake. Add absolute alcohol, a few drops at a time, shaking after each addition until the mixture becomes clear. Allow to stand. Turbidity after 10 min. is an indication of caramel.

## MARSH TEST FOR ARTIFICIAL COLORS—TENTATIVE

(Caramel and Some Coal Tar Dyes)

### 81 REAGENT

*Marsh Reagent.*[11]—Prepare as directed under **79.**

### 82 DETERMINATION

Place 10 cc of the sample in a 20 cc test tube, add sufficient Marsh reagent to nearly fill the tube, and shake several times. Allow the layers to separate; if the lower layer is colored, it is an indication that the sample has been colored with caramel or a coal tar dye.

In the absence of any color, test 10 cc of the sample in the same manner, using sufficient fusel oil, amyl alcohol or pentasol to nearly fill the tube, and shake several times. A deeply colored lower layer is an indication of a coal tar dye; its identity should be confirmed by using the methods under **XXI.**

## DETECTION OF METHANOL IN ALCOHOLIC BEVERAGES[12]

### 83 REAGENTS

(a) *Potassium permanganate soln.*—Dissolve 3 g of $KMnO_4$ in 15 cc of 85% $H_3PO_4$ and make up to 100 cc with $H_2O$.

(b) *Oxalic acid soln.*—Dissolve 5 g of oxalic acid in 100 cc of $H_2SO_4$ (1+1).

(c) *Schiff's reagent.*—Dissolve 0.2 g of Kahlbaum rosaniline hydrochloride in 120 cc of hot $H_2O$, cool, add 2 g of anhydrous $Na_2SO_3$ dissolved in 20 cc of $H_2O$, and 2 cc of HCl, make up to 200 cc, and store in well-filled glass-stoppered amber bottles.

### 84 DETERMINATION

Dilute the alcoholic beverage to 5% total alcohol by volume. Transfer 5 cc of this soln to a 6-inch test tube; add 2 cc of the $KMnO_4$ soln; and let stand 10 min. Remove the excess of $KMnO_4$ by the addition of 2 cc of the oxalic acid soln. As soon as the $KMnO_4$ is decolorized add 5 cc of Schiff's reagent. Mix thoroly and let stand 10 min. If HCHO is present, the characteristic reddish purple color is produced.

Run blanks on pure ethyl alcohol and on ethyl alcohol containing about 1% of methanol.

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# XVIII. COFFEE AND TEA

## GREEN COFFEE

### 1 MACROSCOPIC EXAMINATION—TENTATIVE

A macroscopic examination usually shows the presence of excessive quantities of black and blighted coffee beans, coffee hulls, stones, and other foreign matter. Separate these by hand picking and determine the quantity gravimetrically.

### 2 COLORING MATTERS—TENTATIVE

Shake vigorously 100 g or more of the sample with cold $H_2O$ or alcohol, 70% by volume. Strain thru a coarse sieve and allow to settle. Identify soluble colors in the soln and insoluble pigments in the sediment as directed under **XXI.**

## ROASTED COFFEE

### 3 MACROSCOPIC EXAMINATION—TENTATIVE

Artificial coffee beans are apparent from their regularity of form, and roasted legumes and lumps of chicory in whole roasted coffee can be picked out and identified microscopically. For ground coffee sprinkle some of the sample on cold $H_2O$ and stir lightly. Fragments of pure coffee, if not overroasted, will float, while fragments of chicory, legumes, cereals, etc., will sink immediately, chicory coloring the $H_2O$ a decided brown. In all cases identify the particles that sink by microscopical examination.

### 4 PREPARATION OF SAMPLE—OFFICIAL

Grind the sample to pass thru a sieve having holes 0.5 mm in diameter and preserve in a tightly stoppered bottle.

### 5 MOISTURE—TENTATIVE

Dry 5 g of the sample at the temp. of boiling $H_2O$ under a pressure not to exceed 100 mm of Hg, or at a temp. of 105–110° under atmospheric pressure, for 5 hours and subsequent periods of 1 hour each until constant weight is obtained. For whole coffee, grind rapidly to a coarse powder and without sifting and unnecessary exposure to the air weigh portions for the determination. For ground coffee, sample directly without further grinding.

### 6 SOLUBLE SOLIDS—TENTATIVE

Place 4 g of the sample, prepared as directed under **4,** in a 200 cc flask; add $H_2O$ to the mark; allow the mass to infuse for 8 hours, with occasional shaking; let stand 16 hours longer without shaking; filter; evaporate 50 cc of the filtrate to dryness in a flat-bottomed dish; dry at 100°; cool; and weigh.

### 7 ASH—OFFICIAL

Proceed as directed under **XXVII, 8,** using the sample prepared as directed under **4.**

### 8 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 12,** using the ash obtained under **7.**

### 9 ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 13,** using the filtrate obtained under **8.**

### 10 ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as directed under **XXXIII, 5,** using the ash obtained as directed under **7** or the water-insoluble ash obtained as directed under **8.**

### 11 SOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

Proceed as directed under **II, 7** or **10,** using the soln obtained under **9.**

### 12 INSOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

Boil the insoluble ash obtained as directed under **8** with 25 cc of HCl (1+2), filter, wash thoroly with hot $H_2O$, and determine $P_2O_5$ in combined filtrate and washings as directed under **II, 7** or **10.**

### 13 CHLORIDES—OFFICIAL

Proceed as directed under **XII, 33** or **35,**

## CAFFEINE

### 14 *Power-Chesnut Method*[1]*—Official*

Moisten 10 g of the sample, prepared as directed under **4,** with 95% alcohol; transfer to a Soxhlet or similar extraction apparatus; and extract with 95% alcohol for 8 hours, exercising care to assure complete extraction. Transfer the extract with the aid of hot $H_2O$ to a porcelain dish containing 10 g of heavy MgO in suspension in 100 cc of $H_2O$. (This reagent should meet the U. S. P. requirements.) Evaporate slowly on a steam bath with frequent stirring to a dry, powdery mass. Rub the residue with a pestle into a paste with boiling $H_2O$ and transfer with hot $H_2O$ to a smooth filter, cleaning the dish with a rubber-tipped glass rod. Collect the filtrate in a liter flask marked at 250 cc and wash with boiling $H_2O$ until the filtrate reaches the mark. Add 20 cc of $H_2SO_4$ (1+9) and boil gently for 30 min. with a funnel in the neck of the flask. Cool, filter thru a moistened double paper into a separatory funnel, and wash with small portions of $H_2SO_4$ (1+199). Extract with 6 successive 25 cc portions of $CHCl_3$. Wash the combined $CHCl_3$ extracts in a separatory funnel with 5 cc of 1% KOH soln. Filter the $CHCl_3$ into an Erlenmeyer flask. Wash the KOH soln with 2 portions of $CHCl_3$ of 10 cc each, adding them to the flask, together with the $CHCl_3$ washings of the filter paper. Evaporate or distil on a steam bath to a small volume (10–15 cc), transfer with $CHCl_3$ to a weighed beaker, evaporate carefully, dry for 30 min. at 100°, and weigh. Test the purity of the residue by determining N and multiplying by the factor 3.464.

### 15 *Fendler-Stüber Method (Modified)*[2]*—Tentative*

(Adapted for quick results)

Treat a 10 g sample, prepared as directed under **4,** with 10 cc of $NH_4OH$ (1+2) and 200 g of $CHCl_3$ in a glass-stoppered bottle; shake continuously by machine or hand for 30 min.; and chill in an ice bath. Pour the entire contents of the bottle on a 24 cm folded filter, covering immediately with a watch-glass. Collect the filtrate with the funnel resting directly in the neck of a flask (previously weighed with stopper) and having the flask surrounded with ice. Stopper as soon as the soln ceases to run from the funnel in a continuous stream and weigh. Evaporate on a steam bath, removing the last $CHCl_3$ with a current of air. Digest the residue with 80 cc of hot $H_2O$ for 10 min. on a steam bath, shaking frequently, and let cool. Treat the soln with 20 cc (for roasted) and 10 cc (for unroasted) of 1% $KMnO_4$ soln and let stand 15 min. at room temp., shaking occasionally. Add 2 cc of $H_2O_2$ soln (100 cc of 3% $H_2O_2$, free of acetanilid, plus 1 cc of glacial acetic acid). If the liquid is still red or reddish, add the $H_2O_2$ soln, 1 cc at a time, until the excess of $KMnO_4$ is destroyed. Place the flask on a steam bath for 15 min. and add 0.5 cc portions of the $H_2O_2$ soln until the liquid ceases to become lighter. Cool, and filter by suction thru a Gooch crucible, washing with cold $H_2O$. Transfer the filtrate to a separatory funnel and extract 6 times with 25 cc portions of $CHCl_3$. Evaporate the combined $CHCl_3$ extracts to a small volume, transfer to a weighed beaker, finish evaporation, dry at 100° to constant weight (30 min. is usually sufficient), and weigh the residue as caffeine. The weight of the caffeine, multiplied by 2000 and divided by the weight of the $CHCl_3$ aliquot obtained from the first filtration, equals the percentage of caffeine in the 10 g sample. Test the purity of the residue by determining N and multiplying by the factor 3.464.

### 16 CRUDE FIBER—OFFICIAL

Proceed as directed under **XXVII, 19,** using the sample prepared as directed under **4.**

### 17 STARCH—TENTATIVE

Extract 5 g of the sample, prepared as directed under **4,** on a hardened filter with 5 successive 10 cc portions of ether; wash with small portions of 95% alcohol until a total of 200 cc has passed thru; place the residue in a beaker; and proceed as directed under **XXVII, 25.**

### 18 SUGARS[8]—TENTATIVE

Weigh 10 g of the sample, prepared as directed under **4,** into a 250 cc volumetric flask; add 1 g of powdered $NH_4NaHPO_4$; and proceed as directed under **XXVII, 20–22.** Determine the reduced Cu in the $Cu_2O$ precipitate either volumetrically, as directed under **XXXIV, 41,** or electrolytically, as directed under **XXXIV, 43.**

### 19 PETROLEUM ETHER EXTRACT—OFFICIAL

Dry 2 g of the sample, prepared as directed under **4,** at 100°; extract with petroleum ether (b. p. 35–50°) for 16 hours; evaporate the solvent; dry the residue at 100°; cool; and weigh.

### 20 TOTAL ACIDITY—TENTATIVE

Treat 10 g of the sample, prepared as directed under **4,** with 75 cc of alcohol, 80% by volume, in an Erlenmeyer flask; stopper; and allow to stand 16 hours, shaking occasionally. Filter, and transfer an aliquot of the filtrate (25 cc for green coffee, 10 cc for roasted coffee) to a beaker; dilute to about 100 cc with $H_2O$; and titrate with 0.1 *N* alkali, using phenolphthalein indicator. Express the result as the number of cc of 0.1 *N* alkali required to neutralize the acidity of 100 g of the sample.

### 21 VOLATILE ACIDITY—TENTATIVE

Into a volatile acid apparatus (**XVII, 44**) introduce a few glass beads and over these place 20 g of the unground sample. Add 100 cc of recently boiled $H_2O$, place a sufficient quantity of recently boiled $H_2O$ in the outer flask, and distil until the distillate is no longer acid to litmus paper. (Usually 100 cc of distillate will be collected.) Titrate the distillate with 0.1 *N* alkali, using phenolphthalein indicator. Express the result as the number of cc of 0.1 *N* alkali required to neutralize the acidity of 100 g of the sample.

## COATING AND GLAZING SUBSTANCES

### 22 SUGAR AND DEXTRIN—TENTATIVE

Introduce 100 g of the whole coffee into a beaker; add exactly 300 cc of $H_2O$; stir; and allow to stand 5 min., stirring frequently. Filter thru a dry filter and add carefully to the filtrate sufficient dry Pb-acetate to precipitate all the caffe-tannic acid, avoiding an excess of the reagent. Filter thru a dry filter and remove the Pb from the filtrate by the addition of a slight excess of dry, powdered K-oxalate. Filter thru a dry filter and determine reducing sugars as invert sugar in 50 cc of the filtrate, as directed under **XXXIV, 38.** Invert a 75 cc aliquot of the filtrate as directed under **XXXIV, 23** (**b**). Cool, nearly neutralize with NaOH soln (1+1), dilute to 100 cc, and determine reducing sugars as invert sugar in the resulting soln as directed under **XXXIV, 38.** Measure a 100 cc aliquot of the filtrate into a 200 cc flask, add 10 cc of HCl (sp. gr. 1.125), and hydrolyze as directed under **XXVII, 23.** Cool, neutralize with NaOH soln (1+1), dilute to volume, filter thru a dry filter, and determine reducing sugars as invert sugar in 50 cc of the filtrate as directed under **XXXIV, 38.** Calculate the reducing sugars in each instance to percentage by weight of the original coffee. Calculate sucrose from the reducing sugars before and after inversion as directed under **XXXIV, 28,** and calculate dextrin as follows: Subtract the reducing sugars after inversion from the reducing sugars after hydrolysis and multiply the difference by the factor 0.8605 to convert the result to dextrin.

In some instances the presence of sucrose in the water extract may be verified by polarization. The presence of dextrin in the water extract may be verified by polarization, as directed under **XXXIV, 30,** and by the erythro-dextrin test (**XXXIV, 95**) performed on the water extract previous to clarification with Pb-acetate.

### 23 EGG ALBUMIN AND GELATIN—TENTATIVE

Treat 100 g of the whole coffee with 500 cc of $H_2O$ and allow to stand for 5 min., stirring frequently. Filter, and treat separate portions of the filtrate with (1) a 5% soln of tannic acid, or (2) Millon's reagent (**XX, 15**). Boil a third portion of the filtrate. In the presence of egg albumin a more or less heavy precipitate will be formed in each case. As a confirmatory test, treat an aliquot of the filtrate with an excess of tannic acid soln; add a little salt if necessary to secure flocculation of the precipitate; filter; and, without washing, introduce the paper and its contents into a Kjeldahl flask and determine N. By this method coffee not coated with albumin or gelatin will yield less than 10 mg of N per 100 g of sample.

### 24 CHICORY INFUSION—TENTATIVE

Cover 100–150 g of the whole coffee with $H_2O$; allow to soak 2–3 min., stirring frequently; and drain the aqueous washings thru a coarse sieve. Wash the coffee upon the sieve with about 100 cc of $H_2O$ and centrifugalize the combined washings. Decant the clear liquid from the sediment, which should then be drained almost dry on filter paper. Mount the sediment in chloral hydrate soln [**XXXIII, 20** (**g**)] and examine under the microscope for elements of chicory.

### 25 FATS AND WAXES[4]—TENTATIVE

Treat 100–200 g of the beans with low-boiling petroleum ether for 10 min., pour off the petroleum ether, and repeat the process. Filter the combined extracts, evaporate, and determine the index of refraction and the saponification number of the residue as directed under **XXXI, 8** and **22.**

## TEA

### 26 DUST, STEMS, AND FOREIGN LEAVES—TENTATIVE

Place 1 g of the tea in a 300 cc casserole, add 200 cc of boiling $H_2O$, and allow to stand 15 min. This treatment will cause the leaves to unroll, and they will then be in condition for examination as to form and structure.[5] A macroscopic examination will reveal the presence or absence of dust or stems. Only those stems that remain floating after the leaf is thoroly infused should be regarded as woody stems[6] ("floaters").

### 27 PREPARATION OF SAMPLE—OFFICIAL

Grind the sample to pass thru a sieve having circular openings 0.5 mm in diameter.

### 28 MOISTURE—OFFICIAL

Proceed as directed under **XXVII, 2.**

### 29 WATER EXTRACT[7]—OFFICIAL

To 2 g of the ground sample in a 500 cc volumetric flask add 200 cc of hot $H_2O$ and boil over a low flame for 1 hour, rotating occasionally. Close the flask with a rubber stopper thru which passes a glass tube 30 inches long for a condenser. Boil very slowly so that no steam escapes from the top of the air condenser. Cool, dilute to volume, mix thoroly, and filter thru a dry filter paper. Transfer an aliquot of 50 cc to a weighed dish and evaporate to dryness on a steam bath. Place in the oven, heat at 100° for 1 hour, cool, and weigh.

### 30 ASH—OFFICIAL

Proceed as directed under **XXVII, 8.**

### 31 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 12,** using the ash obtained under **30.**

### 32 ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 13,** using the filtrate obtained under **31.**

### 33 ALKALINITY OF THE INSOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 14,** using the insoluble ash obtained under **31.**

### 34 ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as directed under **XXXIII, 5,** using the total ash obtained as directed under **30,** or the insoluble residue obtained under **31.**

### 35 SOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

Proceed as directed under **II, 7** or **10,** using the soln of soluble ash obtained under **32.**

### 36 INSOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

Proceed as directed under **II, 7** or **10,** using the soln obtained under **33.**

### 37 PETROLEUM ETHER EXTRACT—OFFICIAL

Proceed as directed under **19.**

### 38 PROTEIN—TENTATIVE

Determine N as directed under **II, 19, 22** or **24.** To obtain the percentage of N present as protein, subtract the percentage of N present as caffeine from the percentage of total N. Multiply this result by 6.25 to obtain the percentage of protein.

### 39 CRUDE FIBER—OFFICIAL

Proceed as directed under **XXVII, 19.**

### 40 VOLATILE OIL—TENTATIVE

Add 100 g of tea to 800 cc of $H_2O$, distil, extract the distillate several times with petroleum ether, transfer the combined petroleum ether extracts to a weighed dish, evaporate at room temp., dry in a desiccator, and weigh.

## CAFFEINE

### 41 *Power-Chesnut Method*[8]*—Official*

Proceed as directed under **14.**

### 42 *Bailey-Andrew Method*[9]*—Official*

To 5 g of the sample, prepared as directed under **27,** in a 500 cc volumetric flask, add 10 g of heavy MgO and 200 cc of $H_2O$. Boil gently over a low flame for 2 hours, using a small-bore glass tube 30 inches long as a condenser. Cool, dilute to volume, and filter thru a dry paper. Transfer an aliquot portion of 300 cc, equivalent to 3 g of original material, to an Erlenmeyer flask of 1 liter capacity; add 10 cc of $H_2SO_4$ (1+9); and boil until the volume is reduced to about 100 cc. Filter into a separatory funnel, washing the flask with small portions of $H_2SO_4$ (1+99), and shake 6 times with $CHCl_3$, using 25, 20, 15, 10, 10, 10 cc portions. Treat the combined extracts with 5 cc of a 1% soln of KOH and when the liquids have completely separated draw off the $CHCl_3$ layer into a suitable flask or beaker. Wash the alkaline soln in the separatory funnel with 2 portions of $CHCl_3$, of 10 cc each and unite the washings with the main bulk of extract. Evaporate or distil off the $CHCl_3$ to a small bulk, transfer to a weighed flask, evaporate to dryness, and further dry in an oven at 100° to constant weight. Test the purity of the residue by determining N and multiplying by the factor 3.464. This gives a value for anhydrous caffeine.

## TANNIN[10]—TENTATIVE

### 43 REAGENTS

(a) *Potassium permanganate soln.*— Prepare a soln containing 1.33 g per liter and obtain its equivalent in terms of 0.1 *N* oxalic acid.

(b) 0.1 *N oxalic acid.*

(c) *Indigotine soln.*—Prepare a soln containing 6 g of indigotine (free from indigo blue) and 50 cc of $H_2SO_4$ per liter.

(d) *Gelatin soln.*—Soak 25 g of gelatin for 1 hour in saturated NaCl soln, heat until the gelatin is dissolved, cool, and dilute to 1 liter.

(e) *Acid sodium chloride soln.*—Acidify 975 cc of saturated NaCl soln with 25cc of $H_2SO_4$.

(f) *Powdered kaolin.*

### 44 DETERMINATION

Boil 5 g of the tea for 30 min. with 400 cc of $H_2O$, cool, transfer to a 500 cc volumetric flask, and dilute to the mark. To 10 cc of the infusion (filtered, if not clear), add 25 cc of the indigo carmine soln and about 750 cc of $H_2O$. Add the $KMnO_4$ soln from a buret, a little at a time while stirring, until the color becomes light green, then dropwise until the color changes to bright yellow or to a faint pink at the rim. Designate the number of cc of $KMnO_4$ used as "*a.*"

Mix 100 cc of the clear infusion of tea with 50 cc of the gelatin soln, 100 cc of the acid NaCl soln, and 10 g of the powdered kaolin, and shake several min. in a stoppered flask. After allowing the mixture to settle, decant thru a filter. Mix 25 cc of the filtrate with 25 cc of the indigotine soln and about 750 cc of $H_2O$ and titrate with $KMnO_4$ as before. The number of cc of $KMnO_4$ used subtracted from that obtained above, "*a*," gives the quantity of $KMnO_4$ required to oxidize the tannin. 1 cc of 0.1 *N* oxalic acid = approximately 0.0042 g of tannin (gallotannic acid).

## FACING

### 45 GENERAL—TENTATIVE

(1) Examine the ash obtained as directed under **30** for mineral pigments (cf. **XXI, 1**); (2) shake a quantity of the tea with a large volume of water and remove the leaves by means of a sieve. Allow the insoluble matter in the water portion to settle, filter, and examine the residue on the filter paper for insoluble pigments as directed under **XXI, 1.** Catechu and other soluble substances, if used, will be found in the filtrate.

### 46 PARAFFIN AND WAXY SUBSTANCES—TENTATIVE

Spread a quantity of the tea between two sheets of unglazed white paper and place thereon a hot iron. Any greasy substance will stain the paper.[11]

### 47 PIGMENTS USED FOR COLORING OR FACING[12]—TENTATIVE

Place 60 g of the tea in a 60-mesh, 5 to 6 inch sieve, provided with a top. Sift a small quantity (approximately 0.1 g) of the dust upon a piece of semi-glazed, white paper about 8 by 10 inches. (To obtain the requisite quantity of dust, it is sometimes necessary to rub the leaf gently against the bottom of the sieve.) Place the paper on a plain, firm surface, preferably glass or marble, and crush the dust by pressing firmly upon it a flat steel spatula about 5 inches long. Repeat the crushing process until the tea dust is ground almost to a powder, when particles of coloring matter, if present, become visible as streaks on the paper. Brush off the loose dust and examine the paper by means of a simple lens magnifying 7.5 diameters. Bright light is essential to distinguish these particles and streaks. In many cases the character of the pigment is indicated by the behavior of these streaks when treated with reagents and examined under a microscope. The crushed particles of leaf of either black or green tea appear in such quantity that there is no chance of mistaking them for coloring or facing material. Repeat this test, using black, semi-glazed paper for facings such as talc, gypsum, $BaSO_4$, or clay.

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[12] U. S. Treas. Dept. T. D. 35244, March 23, 1915; Proc. Eighth Intern. Cong. Appl. Chem., **18**, 301 (1912); U. S. Dept. Agr. Misc. Circ. **9**, Reg. 24.

# XIX. CACAO BEAN AND ITS PRODUCTS

## 1 PREPARATION OF SAMPLE—OFFICIAL

Mix powdered products thoroly and preserve in tightly stoppered bottles. Chill sweet or bitter chocolate until it becomes hard and reduce to a finely granular condition by grating or shaving. Mix thoroly and preserve in a tightly stoppered bottle in a cool place.

## MOISTURE

### 2 *Method I—Official**

Proceed as directed under **XXVII, 2.**

### 3 *Method II—Tentative*

Dry 2 g of the sample, prepared as directed under **1**, in a Pt dish in an air oven at 100° to constant weight. An Al dish may be used when the ash is not determined in the same sample. Report the loss in weight as moisture.

## 4 ASH—OFFICIAL

Proceed as directed under **XXVII, 8,** using sufficient sample to contain approximately 1 g of water-, sugar-, and fat-free material.

## 5 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 12,** using the ash obtained under **4.**

## 6 ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 13,** using the filtrate from **5.**

## 7 ALKALINITY OF THE INSOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 14,** using the insoluble ash obtained under **5.**

## 8 ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as directed under **XXXIII, 5,** using the total ash as obtained under **4,** or the water-insoluble residue as obtained under **5.**

## 9 TOTAL NITROGEN—OFFICIAL

Proceed as directed under **II, 19, 22** or **24.**

## 10 CASEIN IN MILK CHOCOLATE—TENTATIVE

It is unnecessary to defat the chocolate. Weigh 10 g of the chocolate, prepared as directed under **1**, into a 500 cc Erlenmeyer flask and add 250 cc of 1% Na-oxalate soln. Heat to boiling and boil gently for a few min.; then cool, add 5 g of $MgCO_3$, and filter. Determine N in 50 cc of this filtrate. Pipet 100 cc of the filtrate into a 200 cc volumetric flask and dilute almost to the mark with $H_2O$. Precipitate the casein by the addition of 2 cc of glacial acetic acid or 1 cc of $H_2SO_4$. Make to volume, shake, filter, and determine N in 100 cc of the filtrate. The difference between the two N determinations gives the N derived from the casein which, multiplied by 6.38, gives the quantity of casein present in 2 g of the sample.

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* See note 7, p. xvii.

### 11 SUCROSE AND LACTOSE—TENTATIVE

Transfer 26 g of the sample, prepared as directed under **1**, to an 8-ounce nursing bottle; add about 100 cc of petroleum ether; and shake 5 min. Centrifugalize until the solvent is clear. Draw off the clear solvent by suction and repeat the treatment with petroleum ether. Place the bottle containing the defatted residue in a warm place until the residual traces of petroleum ether are practically expelled. Add 100 cc of $H_2O$, shake until all the chocolate is loosened from the sides and bottom of the bottle, and then shake for 3 min. longer. Add basic Pb-acetate soln from a buret to complete precipitation and then sufficient $H_2O$ to make the total volume of added liquid 110 cc. Mix thoroly and filter thru a folded paper. Precipitate the excess of Pb with dry, powdered K-oxalate. Pipet one 50 cc portion of the Pb-free filtrate into a 100 cc flask, dilute with $H_2O$ to the mark, mix well, and polarize in a 200 mm tube. Obtain the invert reading as directed under **XXXIV, 23 (b)**. Multiply both the direct and the invert readings by 2 to correct for dilution. From the figures obtained calculate the percentages of sucrose ($S$) and lactose ($L$) by the following formula*:

$$S=\frac{(P-I)\,(110+x)}{143.0-\frac{t}{2}}\text{ and}$$

$$L=\frac{P\left(1.1+\frac{x}{100}\right)-S}{0.79},\text{ in which the value of } x \text{ is obtained from}$$

$$x=\frac{0.2244\;(P-21d)}{1-0.00204\;(P-21d)},\text{ in which the value of } d \text{ is obtained from}$$

$$d=\frac{P-I}{143.0-\frac{t}{2}}.$$

### FAT

### 12 *Method I*[1]*—Official*

Prepare in a Knorr extraction tube a tightly packed mat of asbestos purified as for the determination of crude fiber [**XXVII, 17 (c)**] and carefully freed from coarse pieces. Wash the filter with alcohol, ether, and a little petroleum ether. (*All* petroleum ether used in this determination must be redistilled below 60°.) Weigh 2–3 g of the sample, prepared as directed under **1**, into the tube. Insert the tube into a rubber stopper in a filtering bell-jar connected to the suction thru a two-way stopcock, taking care that no rubber particles adhere to the tip of the stem. Place a weighed 150 cc Erlenmeyer flask at such a height that the tube stem passes thru the neck into the flask. (The stem of the tube should be lengthened if necessary.) Fill the tube to about ⅔ of its capacity with the redistilled petroleum ether, and by means of a rod having a flattened end stir the sample thoroly, taking care to crush all lumps. Let stand 1 min. and drain by suction. Regulate the suction so that the collected solvent in the flask will not boil violently. Add the solvent from a wash-bottle, at the same time turning the tube between thumb and finger so that the sides of the tube are washed down by each addition. Repeat the extractions, with stirring, until the fat is removed. (Ten extractions will usually be sufficient.) Remove the tube with stopper from the bell, wash the traces of fat from the end of the stem with petroleum ether, evaporate the solvent, and dry to constant weight at 100°.

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* The derivation of this formula will be found in Browne's Handbook of Sugar Analyses, 2nd Ed.

The fat-free sample may be used for the crude fiber determination.

### *Method II*[2]—*Tentative*

### 13 REAGENTS

(**a**) *Petroleum ether.*—Redistilled below 60°.

(**b**) *Asbestos.*—Prepare as directed under **XXVII, 17 (c)**, but further wash with alcohol, ether, and petroleum ether.

### 14 DETERMINATION

Weigh accurately about 2 g of the sample, prepared as directed under **1**, and without previous drying stratify the charge in an extraction tube with about 0.5 g of asbestos. Extract with petroleum ether in a continuous extractor for 4 hours. Grind the material, to break up any lumps that may have formed, and re-extract for at least 4 hours. (It is advisable to allow the solvent to run thru the material once completely before applying heat for the continuous extraction.) Collect the petroleum ether extract in a weighed flask, evaporate the solvent, and dry the residue to constant weight at 100°. The extracted residue in the extraction tube may be used for the determination of crude fiber.

### 15 MILK FAT IN MILK CHOCOLATE—TENTATIVE

Estimate the quantity of milk fat in milk chocolate from the following formula based on a Reichert-Meissl number of 0.5 for cacao butter:

$$C=\frac{24A+0.5B}{5}, \text{ in which}$$

$A$ = g of butter fat in 5 g of mixed fat;
$B = 5 - A$ = g of cacao fat in 5 g of mixed fat; and
$C$ = Reichert-Meissl number of extracted fat.

From which the

$$\text{Weight of butter fat in 5 g of mixed fat} = \frac{C-0.5}{4.7}, \text{ and the}$$

$$\text{Percentage of butter fat} = \text{percentage of total fat} \times \frac{C-0.5}{23.5}.$$

## EXAMINATION OF FAT

### 16 SEPARATION AND PREPARATION—TENTATIVE

Separate the fat from 2 to 10 g of sample (depending upon the fat content) by shaking the material with 2 or 3 100 cc portions of ethyl ether. Centrifugalize and decant each portion. Combine the portions in a beaker and drive off most of the ether on a steam bath. Filter the ether extracts thru a dry, folded filter and dry at 100°.

### DETECTION OF COCONUT AND PALM KERNEL OILS IN CACAO BUTTER AND FAT EXTRACTED FROM MILK CHOCOLATE[3]—TENTATIVE

### 17 REAGENTS

(**a**) *Alcoholic potassium hydroxide soln.*—Dissolve 25 g of KOH in 200 cc of alcohol.

(**b**) *Salt soln.*—Prepare a saturated soln from common salt.

### 18 EXAMINATION OF CACAO BUTTER

Saponify 5 g of the sample with 15 cc of the alcoholic KOH soln and evaporate the alcohol on a steam bath. Run a blank on pure cacao butter at the same time. Add 5 cc of $H_2O$ and again evaporate to remove the last trace of alcohol. Dissolve the soap in 100 cc of $H_2O$, cool to room temp., and add, while stirring, 100 cc of the saturated salt soln. Allow to stand for 15 min., stirring several times during this period, and then separate the soap by filtration, using a Büchner funnel. To 100 cc of the filtrate add, while stirring, 100 cc of the saturated salt soln and allow to stand for 15 min. Only a slight precipitate should appear. Filter, add to the filtrate a drop of phenolphthalein indicator, neutralize with HCl (1+3), and then add 0.5 cc of this reagent in excess. If the sample consists of pure cacao butter, the soln when acidified will remain clear. If coconut or palm kernel oil is present, the soln will become turbid or milky.

### 19 EXAMINATION OF FAT EXTRACTED FROM MILK CHOCOLATE

Milk fat, if present in cacao butter subjected to this test, produces a turbidity less in intensity than that produced by the same percentage of coconut or palm kernel oil. For example, cacao butter containing 10%, 15%, or 20% milk fat produces, respectively, no opalescence, a faint opalescence, or an opalescence. For this reason, when the fat to be examined has been extracted from a cacao product that contains lactose or casein, multiply the percentage of lactose in the cacao product by 0.8, or the percentage of casein by 1.1, to obtain the percentage of milk fat in the product, and from this result calculate the percentage of milk fat in the total fat. If this percentage corresponds to 15% or less, a blank of cacao butter containing 15% milk fat may be used; otherwise make up a mixture of cacao butter and milk fat in the proportions indicated by the calculations.

Test the fat extracted from the sample under examination as directed under **18**, but use the prepared mixture of cacao butter and milk fat instead of the pure cacao butter for the blank. If the fat being tested contains coconut oil or palm kernel oil, the last filtrate, when acidified, will be more turbid or milky than the blank.

## CRITICAL TEMPERATURE OF DISSOLUTION IN ACETIC ACID TEST[5]—TENTATIVE

### 20 REAGENTS

(a) *Glacial acetic acid.*—99.5%.
(b) *Potassium hydroxide soln.*—0.1 *N*.

### 21 APPARATUS

Insert a thermometer reading to 0.1° into a cork that fits a 6x¾ inch test tube. The thermometer should extend far enough into the tube so that the bulb will be covered by 10 cc of liquid. Place the test tube in a larger tube (4 x 1¼ inch) containing glycerol and hold firmly in place with a cork having a groove cut in the side to equalize the pressure when heat is applied.

### 22 DETERMINATION

To remove traces of moisture, filter a portion of the sample to be examined thru a dry paper in an oven in which a temp. of about 110° is maintained. Allow the filtered sample to cool until barely warm and weigh 5 g of the sample and 5 g of the acetic acid reagent into the test tube. Insert the cork holding the thermometer and place the test tube in the glycerol bath. Heat, and shake the apparatus frequently until a clear soln of the fat and acetic acid is obtained. Allow the soln to cool with constant shaking without removing from the glycerol bath. Note the temp. at which the first sign of turbidity appears. Make a similar test with the same acetic acid on a sample of pure cacao butter.

Free fatty acids lower the turbidity temp. A correction, therefore, must be made for the acid value of the sample. If the strength of the acetic acid reagent is such that the turbidity temp. of the pure cacao butter is approximately 90°, one unit of acid value will cause a reduction of 1.4° in the critical temp. of dissolution. If the turbidity temp. is approximately 100°, one unit of acid value will cause a reduction of 1.2°. For intermediate temp. the reduction is proportional.

Determine the acid value (mg of KOH required to neutralize the free fatty acids in 1 g of the sample) of both the sample and the pure cacao butter as directed under **XXXI, 32,** using 5 g of fat. Multiply the acid value by the correction factor and add the result to the observed turbidity temp. The figure obtained is the true critical temp. of dissolution. If the true critical temp. of dissolution of the sample is lower than that of the pure cacao butter by more than 3° in the case of fat from chocolate liquors or sweet chocolates, and by more than 6° in the case of fat from milk chocolates, adulteration with coconut, palm kernel, corn, peanut, cottonseed oils, etc., or their stearines, is indicated.

### ACETONE-CARBON TETRACHLORIDE TEST[5]—TENTATIVE

### 23 REAGENT

*Acetone-carbon tetrachloride.*—Mix equal volumes of acetone and $CCl_4$.

### 24 DETERMINATION

Dissolve 5 cc of the warm fat, which has been previously filtered thru a dry filter paper in an oven at about 110° to remove traces of moisture, in 5 cc of the acetone-$CCl_4$ reagent in a test tube. Allow the soln to stand in ice $H_2O$ for 20–30 min. Run a blank on a sample of pure cacao butter at the same time. If hydrogenated oil, tallow, oleostearin, or paraffin is present, a white flocculent precipitate will soon appear. If the $H_2O$ is cold enough, cacao butter may solidify. If a precipitate is formed, remove the sample from the ice $H_2O$ and allow to remain at room temp. for a time. Solidified cacao butter will soon melt and go into soln, but if the precipitate is due to any of the above-mentioned possible adulterants a much longer time will be required.

### 25 MELTING POINT—OFFICIAL

Proceed as directed under **XXXI, 13.** Melting-point determinations upon cacao fat do not become normal until the fat has been kept for at least 24 hours in a cool place.

### 26 INDEX OF REFRACTION—OFFICIAL

Proceed as directed under **XXXI, 8.**

### 27 IODINE ABSORPTION NUMBER—OFFICIAL

Proceed as directed under **XXXI, 18** or **20.**

### 28 SAPONIFICATION NUMBER—OFFICIAL

Proceed as directed under **XXXI, 22.**

### 29 REICHERT-MEISSL AND POLENSKE VALUES[6]—OFFICIAL

Proceed as directed under **XXXI, 26.**

## CACAO SHELL[7]

### 30 REAGENTS

(a) *Chloral hydrate soln.*—Prepare as directed under **XXXIII, 20(g).**

(b) *Acidified chloral hydrate-glycerol soln.*—Prepare as directed under **XXXIII, 20(h).**

### 31 APPARATUS

(a) *Centrifuge or suction filter.*

(b) *Ruled slides.*—Prepare in the laboratory as follows: Warm an ordinary microscopic slide, dip in hot melted beeswax, drain, and cool. Rule with a fine needle and etch with HF. So place the rulings that at any point on the slide two lines are visible in the microscopic field.

(c) *Cover-glasses.*—No. 2, 22 mm square.

(d) *Compound microscope.*—Use a magnification of approximately 120 mm. (A higher magnification is helpful in the case of certain fragments difficult to identify.)

### 32 PREPARATION OF STANDARDS

Prepare standard samples from clean cacao nibs and shells. Grind and thoroly defat both nibs and shells separately until each passes thru a 100-mesh sieve. Weigh, and mix the nib powder and shell powder in desired proportions, finally sieving each standard thru the 100-mesh sieve to insure thoro and uniform mixing.

### 33 PREPARATION OF SAMPLE

(a) *Bitter chocolate and cocoa.*—Completely remove the fat from a portion of the sample, prepared as directed under **1**, by extracting 3 or 4 times with gasoline or ether in a centrifuge or on a suction filter. If necessary, remove the sample to a mortar, grind, and continue the defatting process to completion. Dry, powder, and mix the sample thoroly by passing twice thru a 100-mesh sieve. Weigh 2 mg on a tared ruled slide, or weigh and transfer to a ruled slide and mount with just sufficient chloral hydrate soln or acidified chloral hydrate-glycerol soln to fill in under the cover-glass. Before applying the cover-glass stir and spread the material with the point of a needle to secure a uniform mount. Warm slightly for 15–20 min. (do not boil) and let stand until the tissues have cleared (preferably about 12 hours for the chloral hydrate soln, or 20–30 min. for the acidified chloral hydrate-glycerol soln).

(b) *Sweetened chocolate and cocoa.*—If the sample contains sugar, remove the sugar by washing the defatted material several times with $H_2O$ and finally with a mixture (equal volumes) of alcohol and ether. Dry, powder, and mix the sample thoroly. Weigh 2 mg, transfer to the ruled slide, and proceed as directed under (a).

Since it has been shown that sugar does not interfere with the determination, some analysts prefer not to remove sugar, in which case 5 mg of defatted material is taken and the results calculated to the basis of 2 mg of fat and sugar-free material.

### 34 EXAMINATION

Examine the entire mount and count all fragments of stone cell tissue present. Compare the results with those obtained on standard samples containing known percentages of cacao shell. Report results on the fat-free basis.

### CRUDE FIBER[8]—OFFICIAL, FIRST ACTION

**35** (For cacao products except milk-chocolate.)

Treat 7 g of liquor or 50 g of sweet chocolate in a nursing bottle with 100 cc of ether, centrifugalize, and decant the supernatant liquor twice. Dry the residue in an oven at about 100° and then powder in the bottle with a flattened glass rod. (In some cases it may be necessary to grind the material in a mortar and extract a third time with ether.) Wash the mixture in the nursing bottle with three 100 cc portions of $H_2O$ at room temp., shaking well each time, until no cocoa material adheres to the bottle. Centrifugalize after each washing for 10–15 min., and decant the aqueous layer. Wash the residue in the same fashion with two 100 cc portions of 95% alcohol and one 100 cc portion of ethyl ether. Transfer the residue to a Pt dish, dry to constant weight, and grind in a mortar. Weigh 2 g of the dried material and determine the percentage of crude fiber as directed under **XXVII, 19,** using linen for both acid and alkaline filtrations. Calculate the percentage of crude fiber on moisture-fat- and sugar-free basis ($E$) by the formula $E=0.7D$.

### 36 CRUDE FIBER—TENTATIVE

(For milk chocolate.)

Proceed as directed under **XXVII, 19,** using sufficient sample prepared as directed under **1** to contain approximately 1 g of water-, sugar-, and fat-free material, making both filtrations thru paper and weighing the washed fiber either upon a weighed filter in the usual way, or rinse from the paper into a weighed Gooch, dry, and weigh.

### STARCH

### 37 *Direct Acid Hydrolysis Method—Tentative*

Weigh 4 g of the sample if unsweetened, or 10 g if sweetened, into a small porcelain mortar; add 25 cc of ether; and grind. After the coarser material has settled, decant the ether, together with the fine suspended matter, on an 11 cm paper of sufficiently fine texture to retain the crude starch. Repeat this treatment until no more coarse material remains. After the ether has evaporated from the filter, transfer the fat-free residue to the mortar by means of a jet of cold $H_2O$ and rub to an even paste, filtering on the paper previously used. Repeat this process until all the sugar is removed. In the case of sweetened products the filtrate should measure at least 500 cc. Determine crude starch in the extracted residue as directed under **XXVII, 23.**

### 38 *Diastase Method—Tentative*

Remove fat and sugar from 4 g of the sample if unsweetened, or 10 g if sweetened as directed under **34.** Carefully wash the wet residue into a beaker with 100 cc of $H_2O$, heat to boiling over asbestos with constant stirring, and continue the boiling and stirring for 30 min. Replace the $H_2O$ lost by evaporation and immerse the beaker in a water bath kept at 55–60°. When the liquid has cooled to the temp. of the bath, add 20 cc of freshly prepared malt extract (**XXVII, 26**) and digest the mixture for 2 hours with occasional stirring. Boil a second time for 30 min., dilute, cool, and digest as before with another 20 cc portion of the malt extract. Heat again to boiling, cool, and transfer to a 250 cc flask. Add 3 cc of alumina cream, dilute to the mark and filter thru a dry paper. The residue on the paper should show no signs of starch when examined microscopically. Continue from this point as directed under **XXVII, 25,** beginning with the words "Place 200 cc of the filtrate in a flask, add 20 cc of HCl (sp. gr. 1.125)."

### 39 COLORING MATTERS—TENTATIVE

Proceed as directed under **XXI, 2**(e).

## SELECTED REFERENCES

[1] J. Assoc. Official Agr. Chem., **9**, 46 (1926).
[2] Ibid., **10**, 42 (1927).
[3] Ibid., **11**, 45 (1928).
[4] Ibid., **13**, 45 (1930).
[5] Ibid., **5**, 263 (1921); **7**, 150 (1923).
[6] Ibid., **13**, 43 (1930).
[7] Ibid., **6**, 98 (1922); **8**, 176 (1924).
[8] Ibid., **14** (1931).

# XX. CEREAL FOODS

## WHEAT FLOUR[1]

### 1 DIRECTIONS FOR SAMPLING—OFFICIAL

Sample a number of sacks equivalent to the square root of the number in the lot, but not less than 10, i.e., 10 from 100 or less, 15 from 225, 20 from 400 sacks, etc.

Select the sacks to be sampled according to their exposure in the ratio of 4 from the most exposed, 3 from the next less exposed, 2 from the next, and 1 from the least exposed portion of the lot.

From each sack to be sampled, draw a core from one corner of the top diagonally to the center of the sack by means of a cylindrical, pointed, polished metal trier, $\frac{1}{2}$ inch in diameter, with a slit at least $\frac{1}{3}$ the circumference. Draw a second core from the other top corner to $\frac{1}{2}$ the distance to the center of the sack.

Deliver the 2 cores at once to a clean, dry, air-tight container which has stood open for a few minutes near the lot of flour to be sampled and seal immediately. Use a separate container for each sack sampled. One of the following containers may be used: (1) A pint fruit jar provided with a rubber gasket; (2) a rubber pouch which can be tied or sealed to exclude moisture or air; (3) a tin can or box with a moisture and air-tight friction top.

Before opening the sample for analysis, alternately invert and roll each container 25 times, or more if necessary, to secure a homogeneous mixture. Avoid extreme temp. and humidities when opening the containers for analysis. Keep the sample tightly sealed at all other times.

### TOTAL SOLIDS (MOISTURE, INDIRECT METHOD)

### *I. Vacuum Method*[2]*—Official*

### 2 APPARATUS

(a) *Metal dish.*—Diameter about 55 mm, height about 15 mm, provided with an inverted slip-in cover fitting tightly on inside.

(b) *Air-tight desiccator.*—Should contain reignited quick lime or $CaC_2$.

(c) *Vacuum oven.*—Connect with a pump capable of maintaining a partial vacuum in the oven with a pressure equivalent to 25 mm or less of Hg and provided with a thermometer passing into the oven in such a way that the bulb is near the samples. Connect a $H_2SO_4$ gas drying bottle with the oven for admitting dry air when releasing the vacuum.

(d) *Mercury manometer.*—Used to indicate the pressure of the partial vacuum.

### 3 DETERMINATION

Weigh accurately about 2 g of the well-mixed sample in a covered dish that previously has been dried at 98–100°, cooled in the desiccator, and weighed soon after attaining room temp. Loosen the cover (do not remove) and heat at 98–100° to constant weight (approximately 5 hours) in a partial vacuum having a pressure equivalent to 25 mm or less of Hg. Admit dry air into the oven to bring to atmospheric pressure. Immediately tighten the cover on the dish, transfer to the desiccator, and weigh soon after room temp. is attained. Report the flour residue as total solids and the loss in weight as moisture (indirect method).

### *II. Air-Oven Method*[3]*—Official*

(This method gives results closely approximating those obtained by the vacuum method.)

### 4 APPARATUS

(**a**) *Metal dish and desiccator.*—Described under **2**(**a**).

(**b**) *Oven.*—Maintained at approximately 130° (±3°) and provided with an opening for ventilation.

(**c**) *Thermometer.*—Place with its bulb near the samples.

### 5 DETERMINATION

Weigh accurately approximately 2 g of the well-mixed sample in a covered dish that has been dried previously at approximately 130° (±3°), cooled in the desiccator, and weighed soon after attaining room temp. Uncover the sample and dry the dish, cover, and contents in the oven at approximately 130° (±3°) for 1 hour. Cover the dish while still in the oven, transfer to the desiccator, and weigh soon after room temp. is attained. Report the flour residue as total solids and the loss in weight as moisture (indirect method).

### 6 ASH[4]—OFFICIAL

Weigh 3–5 g of the well-mixed sample into a shallow, relatively broad ashing-dish, which has been ignited, cooled in a desiccator, and weighed soon after attaining room temp. Incinerate in a furnace at approximately 550° (dull red) until a light gray ash results, or until no further loss in weight occurs. Cool in the desiccator and weigh soon after room temp. is attained. Reignited quick lime or $CaC_2$ is a satisfactory drying agent for the desiccator.

### 7 CRUDE FAT OR ETHER EXTRACT—OFFICIAL

Proceed as directed under **XXVII, 15.** With fine flour the addition of an equal weight of clean, dry sand may be necessary.

### 8 FAT (ACID HYDROLYSIS METHOD)[5]—OFFICIAL

Place 2 g of the flour in a 50 cc beaker, add 2 cc of 95% alcohol, and stir so as to moisten all particles. (The moistening of the sample with alcohol prevents lumping on addition of the acid.) Add 10 cc of HCl (25+11), mix well, set the beaker in a water bath held at 70–80°, and stir at frequent intervals for 30–40 min. Add 10 cc of 95% alcohol and cool. Transfer the mixture to a Röhrig or Mojonnier fat extraction apparatus. Rinse the beaker into the extraction tube with 25 cc of ethyl ether in 3 portions and shake the mixture well. Add 25 cc of redistilled petroleum ether (b. p. below 60°) and mix well. Let stand until the upper liquid is practically clear. Draw off as much as possible of the ether-fat soln thru a filter consisting of a pledget of cotton packed just firmly enough in the stem of a funnel to allow free passage of the ether into a weighed 125 cc beaker-flask containing some porcelain chips or broken glass. Before weighing the beaker-flask dry it in a drying oven at 98–105° and then allow it to stand in the air to constant weight. Re-extract the liquid remaining in the tube twice, each time with only 15 cc of each ether. Shake well on the addition of each ether. Draw off the clear ether solns thru the filter into the same flask as before and wash the tip of the spigot, the funnel, and end of the funnel stem with a few cc of a mixture of the 2 ethers in equal volumes free from suspended $H_2O$. Evaporate the ethers slowly on a steam bath, then dry the fat in a drying oven at 90–105° until it ceases to lose weight (approximately 75 min.). Remove the flask from the oven, allow it to stand in the air until no further change in weight takes place, and weigh.

(Owing to the size of the flask and the nature of the material, there is less error by cooling in air than in a desiccator.) Correct this weight by a blank determination on the reagents used.

### 9 CRUDE FIBER—OFFICIAL

Proceed as directed under **XXVII, 17–19.**

### 10 ACIDITY OF WATER EXTRACT—TENTATIVE

Weigh 18 g of the flour into a 500 cc Erlenmeyer flask and add 200 cc of $CO_2$-free $H_2O$. Keep the flask, loosely stoppered, for an hour in a water bath maintained at 40°, shaking occasionally. Filter thru a dry, folded filter, returning the first 10-15 cc of the filtrate to the filter. Titrate 100 cc of the clear filtrate with 0.05 $N$ NaOH soln, using phenolphthalein indicator. 1 cc of 0.05 $N$ NaOH soln $= 0.05\%$ acidity as lactic acid.

### 11 HYDROGEN-ION CONCENTRATION[6]—OFFICIAL, FIRST ACTION

Weigh 10 g of flour (or some multiple thereof) into a clean, dry Erlenmeyer flask and add for each 10 g of flour 100 cc of distilled $H_2O$ at a temp. of 25°. Shake or whirl the flask until the particles of flour are evenly suspended and the mixture is free from lumps. Place in a thermostat at 25° and shake, continuously or intermittently in such a manner as to keep the flour particles in suspension, for 30 min. Let stand quietly for 10 min., then decant the supernatant liquid into a suitable vessel and immediately determine its hydrogen-ion concentration electrometrically, using electrodes and a potentiometric set-up that have been checked thru the use of a buffer soln of known hydrogen-ion concentration.

### 12 SUGARS—TENTATIVE

Determine reducing sugars and sucrose as directed under **XXVII, 21** and **22.**

### PROTEIN—OFFICIAL

### 13 (Organic and Ammoniacal Nitrogen)

Determine N as directed under **II, 19, 22** or **24,** and multiply the percentage of N by 5.7 to obtain the percentage of protein. Also use the factor 5.7 to convert N to protein in wheat used for manufacturing purposes or human food.

### 70 PER CENT ALCOHOL–SOLUBLE PROTEINS

### 14 *I. By Nitrogen Determination—Tentative*

Transfer 4 g of the flour to a 150–200 cc bottle or Erlenmeyer flask and add 100 cc of alcohol, 70% by volume, taking care that none of the material adheres to the bottom of the container. Shake thoroly 10–12 times at intervals of 30 min. at room temp., or shake continuously in a shaking machine for 1 hour, and then set aside overnight. Shake thoroly once more and filter thru a dry, folded filter, returning the first runnings to the filter until a clear filtrate is obtained. Pipet 50 cc of the filtrate, equivalent to 2 g of the sample, into a Kjeldahl flask; dilute with 100 cc of $H_2O$ to prevent frothing during digestion; and determine N as directed under **II, 19, 22** or **24.** (Make a blank determination on the reagents.)

### *II. By Polarization—Tentative*

### 15 REAGENT

*Millon's reagent.*—Dissolve metallic Hg in an equal weight of $HNO_3$ and dilute the soln with an equal volume of $H_2O$. The freshly prepared soln must be used.

### 16 DETERMINATION

Weigh 15.97 g of the flour into a 300 cc flask and add 100 cc of alcohol (sp. gr. 0.90). Shake at 30 min. intervals for 3 hours and then let stand overnight. Filter thru a dry, folded filter and polarize in a 200 mm tube. Precipitate the proteins in 50 cc of the filtrate by the addition of 5 cc of Millon's reagent. Shake, filter, and polarize the filtrate in a 200 mm tube. Multiply the reading in degrees Ventzke by 1.1 to correct for the dilution and deduct the product from the first reading. This difference, multiplied by 0.2, gives the percentage of gliadin N. [7]

### 17 PROTEINS SOLUBLE IN 5 PER CENT POTASSIUM SULFATE SOLUTION—TENTATIVE

Weigh 6 g of the flour into a 200 cc flask and introduce exactly 100 cc of 5% $K_2SO_4$ soln. Shake at 30 min. intervals for 3 hours or, better, agitate at moderate speed in a mechanical shaker for 1 hour; let settle 30 min.; and filter. Determine the N in 50 cc of the filtrate as directed under **II, 22** or **24**, making allowance for the N contained in the reagents.

### 18 GLOBULIN AND ALBUMIN (EDESTIN AND LEUCOSIN) AND AMINO NITROGEN—TENTATIVE

Weigh 10 g of the flour into a 500 cc Erlenmeyer flask, add 250 cc of 1% NaCl soln, stopper the flask, and shake thoroly. Let stand, with occasional shaking, for 3 hours; filter; and evaporate 100 cc of the filtrate to a small volume in a Kjeldahl digestion flask with 5 cc of $H_2SO_4$. Add 25 cc more acid and determine the N as directed under **19, 22,** or **24.** To a second 100 cc of the filtrate add 5 cc of 20% phosphotungstic acid soln, shake thoroly, allow to settle, and filter by decantation. Wash slightly with $H_2O$, concentrate the filtrate with 5 cc of $H_2SO_4$ in a Kjeldahl flask, and determine the amino N as directed under **II, 19, 22,** or **24.** Deduct the amino N from N found in the first fraction to obtain the N as globulin and albumin[8]. Make allowance for the N contained in the reagents.

### GLUTENIN

### 19 *Method I.—Tentative*

Deduct the sum of the $K_2SO_4$-soluble N, **17,** and the alcohol-soluble N, **14,** from the total organic and ammoniacal N, **13,** and multiply the difference by 5.7.

### *Method II.—Tentative*[9]

### 20 REAGENTS

(a) *Barium hydroxide.*—Freshly powdered.

(b) *Methyl alcohol.*—96%; free from acids, aldehydes, and ketones. Synthetic methanol preferred.

Flour and reagents should be allowed a minimum exposure to the air at all times.

### 21 DETERMINATION

Weigh 8 g of flour into a 200 cc flask, preferably a sugar flask or one that readily permits thoro mixing of the suspension when shaken. Add 0.2 g of $Ba(OH)_2$, follow at once with 50 cc of distilled $H_2O$ ($CO_2$-free), and stopper tightly. Shake immediately to form a smooth suspension. Let stand for 1 hour at room temp., shaking frequently. Add sufficient methyl alcohol to allow 5 cc of liquid above the mark (to correct for volume of flour) when thoroly mixed. Shake vigorously for 2 min. After the starch settles to the bottom, pour the supernatant liquid *at once* thru a cotton plug, repeating the filtrations 2 or 3 times if necessary. Immediately withdraw 50 cc for the Kjeldahl N determination. Do not allow more than 15 min. to elapse from the time the methyl alcohol is added to the withdrawal of the 50 cc aliquot, because gliadin will begin to precipitate after standing for a short period of time. To prevent troublesome foaming add 150–200 cc of $H_2O$ to the Kjeldahl flask before starting the digestion of the alcoholic extract. Convert the N to protein by the factor 5.7, subtract the percentage of protein in the extract from the percentage of total protein ($N \times 5.7$) as determined in a separate portion of flour, and record the difference as the percentage of glutenin in the flour.

### CRUDE GLUTEN

**22** *Qualitative Test*[10]*—Tentative*

Place a very small quantity (about 1.5 mg) of the flour on a microscope slide; add a drop of water containing 0.2 g of water-soluble eosin in 1 liter; and mix by means of a cover-glass, holding it at first in such a manner that it is raised slightly above the slide and taking care that none of the flour escapes from beneath it. Finally allow the cover-glass to rest on the slide and rub it back and forth until the gluten has collected into rolls. The operation should be carried out on a white paper so that the formation of gluten rolls can be noted. Wheat flour, or other flours containing gluten, show by this treatment a copious quantity of gluten, which absorbs the eosin with avidity, assuming a carmine color. Rye flour and corn flour yield only a trace of gluten; buckwheat flour, no appreciable quantity. If the flour is coarse or contains a considerable quantity of bran elements, as is true of buckwheat flour and low-grade wheat flour, the test should be made after bolting as the bran particles and coarse lumps interfere with the formation of gluten rolls.

**23** *Quantitative Method—Tentative*

(Results are approximate)

Weigh 25 g of the flour into a cup or porcelain mortar; add sufficient tap $H_2O$ (about 15 cc) to form a firm dough ball; and work into a dough with a spatula or pestle, taking care that none of the material adheres to the utensil. After allowing the dough to stand in $H_2O$ at room temp. for an hour, knead gently in a stream of tap $H_2O$ until the starch and all soluble matters are removed. Do this operation, which requires approximately 12 min., over bolting cloth. To determine whether or not the gluten is starch-free let 1 or 2 drops of the wash $H_2O$, obtained by squeezing the gluten, fall into a beaker containing perfectly clear water. If starch is present a cloudiness appears. Allow the gluten thus obtained to stand in $H_2O$ for an hour; press as dry as possible between the hands; roll into a ball; place in a weighed, flat-bottomed dish; and weigh as moist gluten. Transfer to an oven, dry to constant weight at 100° (about 24 hours), cool, and weigh as dry gluten; or heat the moist gluten at approximately 230° for 15–20 min., or until the puffed gluten-ball has become firm. Then dry to constant weight in a drying oven.

### WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL[11]—TENTATIVE

**24** REAGENTS

(a) *Alcohol.*—40%. Mix 50 volumes of $H_2O$ and 35 volumes of 95% alcohol.
(b) *Asbestos.*—Ignite and rub thru an 8-mesh sieve.

**25** DETERMINATION

Place 20 g of the sample in an 8-oz nursing bottle, add 100 cc of $H_2O$ from a pipet, shake the bottle to prevent lumping of the sample, and add exactly 100 cc more $H_2O$. Mix the contents of the stoppered bottle gently by hand or on a slowly revolving wheel for 1 hour. (The temp. of the $H_2O$ should not exceed 30°.) Centrifugalize to facilitate filtration and filter thru a thin asbestos pad in a Hirsch funnel, using light suction. Determine N in 50 cc of the filtrate as directed under **II, 19, 22** or **24,** distilling the $NH_3$ into 20 cc of 0.1 *N* acid. Run a blank on the reagents. Pipet off 100 cc of the above filtrate into a 200 cc volumetric flask, add 15 cc of NaCl soln (28 g diluted to 300 cc), fill nearly to the mark with 95% alcohol, mix carefully to avoid foaming, cool to room temp., make up to the mark with alcohol, mix well, and allow to stand overnight. Pipet off the supernatant liquid and filter thru an 18½ cm fluted filter paper. Determine N in 100 cc of the filtrate as above. (In order to avoid bumping, it is advisable to add the $H_2SO_4$ and boil off the alcohol before adding the $K_2SO_4$ and HgO.) Subtract the value obtained from the water-soluble N to obtain the water-soluble N precipitable by 40% alcohol.

### 26 LIPOIDS[12]—OFFICIAL

Add 15 cc of alcohol, 70% by volume, to 5 g of the flour in a 200 cc nursing bottle. Give the bottle a gentle rotary motion so as to moisten all the particles with the alcohol, stopper, and set in a water bath kept at 75–80°. Heat for 15 min. with frequent mixing by the same rotary motion. Add 27 cc of 95% alcohol, stopper the bottle, and shake vigorously for 2 min. Cool, add 45 cc of ether, and shake well for 5 min. (The sample should now be in a fine state of division.) Centrifugalize just sufficiently to throw the solid particles out of suspension but not so as to pack the sample too firmly. Decant the liquid into a 250 cc beaker containing some bits of broken porcelain or glass, and rinse off the bottle neck with ether. Re-extract the sample with 3 successive 20 cc portions of ether, shake 1 or 2 min. each time, centrifugalize, and decant into the beaker containing the first extract. Evaporate the combined ether-alcohol extracts just to dryness on the steam bath. Drive off any remaining moisture on the sides of the beaker by placing in a drying oven at 98–105° for 5 min. Dissolve the dry extract in approximately 15 cc of $CHCl_3$ and filter the soln into a previously dried and weighed Pt dish thru a pledget of cotton packed in the stem of a funnel. Free with a glass rod any solid extract adhering to the beaker and transfer thru the filter into the first washings by means of $CHCl_3$ from a wash bottle all extract from the beaker bottom and sides. Finally wash the funnel and stem tip. The filtrate should be perfectly clear. Evaporate the $CHCl_3$ on a steam bath and dry the dish and contents in a drying oven at 98–105° until no more weight is lost (75–90 min.). Weigh. Report the extract as lipoids.

### 27 LIPOID PHOSPHORIC ACID[13] ($P_2O_5$)—OFFICIAL

Dissolve the lipoids in 5–10 cc of $CHCl_3$, add 5–10 cc of 4% alcoholic KOH soln, evaporate to dryness on a steam bath, and char well in a furnace at a faint red heat. Cover the dish with a cover-glass, add sufficient $HNO_3$ (1+9) to make the soln slightly acid, warm on a steam bath, and filter. Wash the residue and filter well with hot $H_2O$. Determine $P_2O_5$ in the filtrate as directed under **II, 7** or **10.** Report as lipoid $P_2O_5$.

## UNSAPONIFIABLE RESIDUE[14]

### *I. F.A.C. Method—Tentative*

### 28 REAGENT

*Petroleum ether.*—Redistilled below 75°. Make a blank determination by evaporating 350 cc of the reagent with about 0.25 g of stearine or other hard fat (previously brought to constant weight by heating) and drying as in the actual determination. The blank must not exceed a few mg.

### 29 APPARATUS

*Extraction cylinder.*—Glass-stoppered, graduated at 40 cc, 80 cc, and 130 cc, and of the following dimensions: diameter about 1⅜ inches, height about 12 inches.

### 30 DETERMINATION

Place the extract from 5 g of flour prepared as directed under **26** in a 200 cc Erlenmeyer flask, add 5 cc of 50% aqueous KOH, and boil the mixture for 1 hour under a reflux condenser. Transfer to the extraction cylinder and wash to the 40 cc mark with redistilled 95% alcohol. Complete the transfer, first with warm, then with cold $H_2O$, until the total volume is 80 cc. Rinse the flask with 50 cc of petroleum ether and add the rinsings to the contents of the cylinder previously cooled to room temp. Shake as vigorously as possible for 1 min. and allow to settle until both layers are clear, when the volume of the upper layer should be about 40 cc. Draw off the petroleum ether layer as closely as possible by means of a slender glass siphon into a separatory funnel of 500 cc capacity. Repeat the extraction at least 6 more times, using 50 cc of petroleum ether for each extraction. Wash the combined extracts into a separatory funnel 3 times with 25 cc portions of 10% alcohol by volume, shaking vigorously each time. Transfer the petroleum ether extract to a weighed Erlenmeyer flask (the flask should be dried at 100-105°, cooled in a desiccator, then brought to equilibrium with the atmosphere and weighed); distil, or if desired, evaporate the petroleum ether on a steam bath in a current of air. Heat the flask (lying on its side) with residue until a constant weight is obtained in an oven at a uniform temp. not less than 100° nor more than 110°. (It is important to displace with air any residue vapors of petroleum ether remaining in the flask after heating and before it is weighed.) Deduct any blank from the weight before calculating unsaponifiable matter. Test the final residue for solubility in 50 cc of petroleum ether at room temp. Filter, and wash free from the insoluble residue, if any. Evaporate and dry in the same manner as before.

## *II. Modified Kerr-Sorber Method*[15]*—Tentative*

### 31 REAGENTS AND APPARATUS

(**a**) *Potassium hydroxide soln*—100 g of KOH dissolved in 100 cc of $H_2O$.

(**b**) *Potassium hydroxide soln.*—Approximately 0.2 *N*. 11.2 g of KOH dissolved in 1000 cc of $H_2O$.

(**c**) *Ethyl alcohol.*—Approximately 95% by volume.

(**d**) *Ethyl ether.*—U.S.P.

(**e**) *Phenolphthalein soln.*—1 g of phenolphthalein dissolved in 100 cc of 95% alcohol.

### 32 APPARATUS

(**a**) *Separatory funnel.*—500 cc capacity, ether-tight. Lubricate the glass connections with $H_2O$.

(**b**) *Erlenmeyer flask or beaker-flask for saponification.*—100-200 cc capacity.

(**c**) *Erlenmeyer flask or beaker-flask.*—250 cc capacity.

### 33 PROCEDURE

Place the extract from 5 g of flour prepared as directed under **26** in the saponification flask. Add 3 cc of the concentrated KOH soln. Place a small, short-stemmed funnel in the neck of the flask to serve as a condenser. Boil gently on the steam bath for about 20 min., or until complete saponification occurs. Cool to about 30°, add 50 cc of ether, mix, and transfer to the separatory funnel. Rinse the flask with 2 successive 50 cc portions of ether, add to the separatory funnel, and mix thoroly. Wash the saponification flask (b) with 100 cc of the dilute KOH soln and pour into the separatory funnel in a slow, steady stream. Rotate the funnel very gently to secure better contact of the solns but do not shake. (Shaking at this stage brings about stubborn emulsion.) Allow the liquids to separate completely and then slowly draw off as much of the soap soln as possible. Do not draw off any layer of emulsion that may be formed. Keep the volume of the ether at about 150 cc by replacing that dissolved by the wash solns. Further treat the ether soln with 2 successive 100 cc portions of the alkaline wash soln in the manner described previously. Add 30 cc of $H_2O$ to the ether and rapidly rotate the liquid layers. When the layers have separated completely, draw off the $H_2O$, repeating this treatment until the washings are free from alkali, as shown by testing with phenolphthalein. (Three washings usually suffice.) Transfer the ether soln quantitatively thru a pledget of cotton in the stem of a funnel to the weighed 250 cc Erlenmeyer flask or beaker-flask. Before weighing the flask dry it in an oven at about 100°, and then allow it to stand in the air to constant weight. Distil off the ether and dry the flask and residue at about 100° until no further loss in weight occurs. Allow the flask with unsaponifiable matter to come to equilibrium with the atmosphere before weighing. Deduct from the weight of the unsaponifiable matter any blank obtained from the reagents used.

### 34 COLD WATER-SOLUBLE EXTRACT—TENTATIVE

Weigh 20 g of the flour into a 500 cc Erlenmeyer flask and add gradually 200 cc of $H_2O$ at a temp. not higher than 10°. Shake vigorously when about 50 cc of $H_2O$ has been added and continue shaking during the addition of the remainder. Allow to stand at 10° for 40 min., shaking occasionally. Filter rapidly, returning the first runnings to the filter, until a clear filtrate is obtained. Pipet 20 cc of the clear filtrate into a weighed dish, evaporate to dryness on a steam bath, and dry in an oven at about 100° for periods of 30 min. to constant weight.

## STARCH[16]—TENTATIVE

### 35 REAGENT

*Hydrochloric acid.*—Mix approximately equal volumes of HCl and $H_2O$ and adjust so that 100 cc of this mixture contains 20.5–21.0 g of HCl.

### 36 DETERMINATION

Weigh accurately a sufficient quantity of the finely ground sample to represent 0.5–1.0 g of starch. Transfer to a funnel fitted with a 9 cm, S. and S. 589 white ribbon or Whatman No. 40 filter paper and extract 4 times with each of the following solvents in the order named: ether, 70% (by volume) alcohol, and water. Transfer the drained filter and contents to a 50 cc beaker. Add 1 cc of the cold HCl reagent; tamp the material with a stirring rod having a flattened end; and continue adding the acid gradually, with constant tamping and stirring, until the filter paper and sample are disintegrated to a smooth suspension. Transfer to a 100 cc wide-mouthed volumetric flask, rinsing with the HCl reagent from a wash bottle. Fill to the mark with the same acid and then add 0.5 cc more to compensate for the volume occupied by the filter paper. Mix, and allow to stand for 3–5 min., shaking occasionally. During the treatment with acid the temp. should not exceed 22°. Filter thru a Gooch crucible prepared with a dry mat of ignited asbestos and filled $\frac{2}{3}$ full with dry, fluffy, ignited asbestos. Pipet 50 cc of the filtrate into a 200 cc beaker (tall form) containing 115 cc of 95% (by volume) alcohol. (To prevent hydrolysis this last step must be completed within 35 min. of the initial contact of the acid with the starch.) Allow the pipet to deliver completely and then stir with a whipping motion for 1 min. to flocculate the precipitated starch. Allow to stand for not longer than 5 min. and then decant the supernatant liquid, which is somewhat turbid, thru a weighed Gooch crucible that has been fitted with a thin pad of ignited asbestos. Wash the precipitate by decantation, using two successive 15 cc portions each of 70% (by volume) and 95% (by volume) alcohol, breaking up the precipitate with a stirring rod during each washing. Decant each portion thru the crucible and finally transfer the starch completely by means of a jet of 95% (by volume) alcohol. Dry the crucible and contents to constant weight, which requires about 1½ hours at 130° or 5 hours in a vacuum oven at about 100°. Allow the crucible to remain uncovered during drying but replace the cover before removing the crucible from the oven, because the starch is extremely hygroscopic. Dry in a desiccator charged with $P_2O_5$, $CaC_2$, or ignited CaO, cool, and weigh immediately.

### CHLORINE

**37** *Qualitative Test (Chlorine-Bleached Flours)—Tentative*

Extract 30 g of the flour with gasoline and allow the solvent to evaporate. A small quantity of oil remains. Heat a piece of Cu wire in a colorless gas flame until it is black and no longer colors the flame green. Dip the hot end of the wire into the oil and again bring into the flame. If Cl or Br has been used as a bleaching agent, a green or blue coloration is produced.

## QUANTITATIVE METHODS

**38** *Method I—Tentative*

Weigh 20 g of the flour into a flat-bottomed Al dish, 8–10 cm in diameter, and dry 5 hours in a drying oven at 98–105°; transfer, with as little exposure to the air as possible, to a continuous fat extractor; and extract for 16 hours with anhydrous alcohol-free ether that is also free from Cl. Transfer the ether extract to a Pt dish and add 25 cc of a soln containing 25 g of NaOH or KOH and 15 g of $NaNO_3$ per liter. Place the dish on a steam bath, evaporate to dryness, and ignite in a muffle at a dull red heat until the contents are thoroly charred. Extract the charred mass with 25 cc of 1% $HNO_3$ and filter. Return the residue to the dish, char, and again extract with 25 cc of 1% $HNO_3$. Filter, wash with hot $H_2O$, return to the dish, and ignite to a white ash. Dissolve the ash in 5% $HNO_3$ and add the soln to the filtrates previously obtained. Determine the Cl in the combined filtrates either gravimetrically, as directed under **XII, 33,** or volumetrically, as directed under **XII, 35,** using 0.02 *N* solns for greater accuracy.

Special precautions should be taken that the air of the laboratory during the entire operation is not contaminated by Cl or HCl fumes and that all reagents employed are as free as possible from Cl. In all cases a blank determination should be conducted at the same time and a correction introduced if necessary. Report results in parts of Cl per million of flour.

*Method II[17]—Tentative*

**39** REAGENTS

(a) *Petroleum ether fractioned at* 60–100°.—Do not use low-boiling petroleum ether because of errors introduced thru rapid evaporation.

(b) *Alcoholic sodium hydroxide soln.*—Dissolve 40 g of NaOH in 1 liter of 95% alcohol.

(c) *Potassium chromate indicator.*—Dissolve 5 g of $K_2CrO_4$ in $H_2O$, add the $AgNO_3$ soln (d) until a slight red precipitate is produced, filter, and dilute to 100 cc.

(d) *Standard silver nitrate soln.*—Dissolve 4.791 g of $AgNO_3$ in $H_2O$ and dilute to 1 liter. 1 cc = 1 mg of Cl. Check by titration against a standardized soln of NaCl.

### 40 DETERMINATION

Weigh 75 g of the flour into a cork-stoppered bottle and add from a pipet 150 cc of the petroleum ether. Stopper tightly and shake vigorously for about 1 min. Allow to stand 1 hour, again shake until the flour particles are in suspension, and then set aside overnight. Shake once more to suspend the flour particles, allow to settle for a few min., and filter thru a dry folded filter. (The funnel and receiving flask should be covered to reduce evaporation during filtration.) Pipet 50 cc of the filtrate into a Pt dish of about 80 cc capacity. If a Pt dish of this size is not available, evaporate the 50 cc of filtrate to a small volume in a porcelain evaporating dish on a steam bath and carefully transfer the fatty concentrate to a small Pt dish, washing out the last traces of fat with several portions of petroleum ether. Add 5 cc of the alcoholic NaOH soln and evaporate to dryness on a steam bath. Char carefully in a muffle at low redness. Extract the charred mass with 2 successive 20 cc portions of $HNO_3$ (1+3), being careful to avoid mechanical losses due to evolution of $CO_2$. Filter these extracts thru a 7 cm quantitative filter paper into a 300 cc flask. Then extract the mass 2 or 3 times with $H_2O$, filtering each portion thru the same 7 cm paper. Return this filter paper to the Pt dish containing the charred residue and ignite to a white ash in a muffle furnace. Dissolve the ash in $HNO_3$ (1+16) and add to the soln already obtained. Neutralize the acidity with a slight excess of dry $CaCO_3$, add 5 cc of the $K_2CrO_4$ indicator, and titrate with the standard $AgNO_3$ soln. At the same time and under the same conditions prepare and conduct a blank containing the quantity of all reagents used in the determination. Since $CaCO_3$ commonly contains appreciable quantities of chlorides, a definite weighed quantity of this reagent should be employed in each determination and the same quantity used in the blank. Correct the buret reading by the number of cc of the standard $AgNO_3$ soln required to give in the blank the shade obtained at the end of the titration of the sample, using in both sample and blank 5 cc of the $K_2CrO_4$ indicator. Report results in parts of Cl per million of flour.

Since the quantity of Cl involved in this determination is relatively small, care should be taken to insure that the laboratory atmosphere is as free from Cl as possible.

## *Method III[18]—Tentative*

### 41 REAGENTS

(a) *Alcohol.*—70% by volume. Mix 73 volumes of 95% alcohol with 27 volumes of $H_2O$.

(b) *Alcohol.*—95% by volume.

(c) *Ethyl ether.*

(d) *Petroleum ether.*

(e) *Alcoholic soda.*—To 95% alcohol add metallic Na cut into small pieces in the proportion of 40 g of Na to 1,000 g of alcohol.

(f) *Nitric acid.*—(2+1). Dilute 500 cc of strong acid to 750 cc with $H_2O$.

(g) *Silver nitrate soln.*—0.005 *N*.

(h) *Potassium thiocyanate.*—0.005 *N*.

(i) *Ferric-ammonium alum soln.*—To a cold saturated soln of ferric-ammonium alum add enough $HNO_3$ to cause the disappearance of the brown color.

**42** DETERMINATION

Weigh 20 g of flour into a 500 cc Erlenmeyer flask, add 60 cc of 70% alcohol, place the flask upon a steam bath, and heat gently (water should steam but not boil), at the same time rotating the flask until the flour and liquid form a uniform mixture. Add 60 cc of 95% alcohol. Stopper the flask and shake thoroly for 2 min. Allow to cool. Add 75 cc of ethyl ether and shake the flask thoroly, then add 150 cc of petroleum ether, and again shake the flask thoroly. Pour the entire liquid contents into a separatory funnel, being careful to avoid, as far as possible, transference of any flour particles. Add to the flask containing the flour, 40 cc of petroleum ether, shake thoroly, and pour the contents into the separatory funnel. Repeat with another 40 cc portion of petroleum ether. Wash the solvents twice with $H_2O$, using 30 cc of $H_2O$ the first time and 10–12 cc the second time; shake thoroly each time, and allow to stand until two sharply defined layers of liquid are formed. Run the washed solvents into a large evaporating dish (or beaker), add 10 cc of the alcoholic soda soln, and evaporate to about 10–15 cc. Pour this liquid into a 50 cc Pt dish and wash out the evaporating dish with small portions of 95% alcohol until all the liquid and residue have been transferred to the Pt dish. Evaporate the contents of the dish to dryness on the steam bath and place the dish with the residue over a small yellow flame of a Bunsen burner. Char the residue but do not heat even to low redness because the alkali may react with the Pt. Allow to cool and add a small quantity of $H_2O$ and 5 cc of $HNO_3$ (2+1). Boil, and then pour thru an ashless filter paper (12½ cm in diameter), catching the filtrate in a sugar flask calibrated at 100 cc and 110 cc. Again boil the residue with a small quantity of $H_2O$ and filter. Remove the filter paper, fold once and place in a Pt dish, and heat to low redness until practically all the paper and residue have been reduced to a gray ash. (Apply low heat to prevent volatilization of chlorides.) Add a small quantity of $H_2O$ and 2.5 cc of $HNO_3$, boil, and filter. Again boil once or twice with small quantities of $H_2O$, filtering as before. Add to the liquid in the sugar flask 25 cc of 0.005 *N* $AgNO_3$ soln, add $H_2O$ to bring the liquid approximately to the 100 cc mark, and place the flask in boiling $H_2O$ for about 5 min. Remove, and allow to cool to room temp. Bring the liquid exactly to the 110 cc mark by adding $H_2O$, stopper the flask, and mix the contents well. Filter thru a dry, fine-pore filter paper (12.5 cm in diameter), and return the first portion of the filtrate to the original soln. Continue to refilter until the filtrate is entirely clear, and thus secure 100 cc of filtrate. Transfer the entire 100 cc to a white porcelain casserole, add 2 cc of ferric-ammonium alum soln, and titrate with 0.005 *N* KCNS until a permanent light brown coloration appears. Deduct the blank determined on all the reagents used and calculate results to the dry basis.

## NITRITE NITROGEN—TENTATIVE

**43** REAGENTS

(**a**) *Sulfanilic acid soln.*—Dissolve 0.5 g of sulfanilic acid in 150 cc of 20% acetic acid.

(**b**) *Alpha-naphthylamine hydrochloride soln.*—Dissolve, by heating, 0.2 g of the salt in 150 cc of 20% acetic acid.

(**c**) *Standard nitrite soln.*—Dissolve 0.1097 g of dry $AgNO_2$ in about 20 cc of hot $H_2O$, add 0.10 g of NaCl, shake until the AgCl flocculates, and dilute to 1 liter. Draw off 10 cc of the clear soln and dilute to 1 liter. 1 cc of the last soln = 0.0001 mg of N as nitrite [cf. **XXXVII, 14 (c)**].

The $AgNO_2$ may be prepared as follows: To a cold soln of about 2 g of $NaNO_2$ or $KNO_2$ in 50 cc of $H_2O$, add a soln of $AgNO_3$ so long as a precipitate forms. Decant the liquid and thoroly wash the precipitate with cold $H_2O$. Dissolve in boiling $H_2O$.

On cooling, the $AgNO_2$ crystallizes out. Dry the crystals in the dark at ordinary temp. (preferably in a vacuum).

### 44 DETERMINATION

(1) Select a series of 100 cc volumetric flasks of uniform dimensions and color and place 2 g of high-grade, nitrite-free flour in each flask; add approximately 70 cc of nitrite-free $H_2O$ and shake until the flour is thoroly moistened. Add to these flasks varying quantities of the standard $NaNO_2$ soln, so that a series of comparison standards will be obtained having a range covering the probable nitrite content of the unknown sample. Reserve one flask for a blank test. In order to avoid making a large series of standards it is well to make a preliminary test to ascertain the approximate nitrite content of the unknown. If the quantity of nitrite present is small, the nitrite soln in the flasks may be increased by 0.4 cc each. If bleaching is excessive, 1 g of flour may be used thruout, or the standards may be given a wider variation in nitrite content.

To each of 2 similar flasks add 2 g of the flour and 90 cc of $H_2O$; shake thoroly; digest all the flasks, including the blank, in a water bath at 40° for at least 15 min.; and add 2 cc each of the sulfanilic acid and alpha-naphthylamine hydrochloride solns to each flask, shaking the mixture after the addition of each reagent. Continue the digestion at 40° for an additional 20 min. The color must be developed in all the flasks under conditions as nearly uniform as possible. Make up to the marks with nitrite-free $H_2O$ and compare the unknown with the series of standards. This may be done in a large, white-enameled pan, the effect of the turbidity due to the flour being minimized by the white background. The solns should be allowed to subside and should not be shaken during comparison; or,

(2) Weigh 20 g of the flour into a 500 cc Erlenmeyer flask; add 200 cc of nitrite-free $H_2O$, previously warmed to 40°; and close the flask with a rubber stopper. Shake vigorously for 5 min. and digest for 1 hour in a water bath, keeping the temp. of the liquid in the flask at 40° and shaking at 10 min. intervals. Finally filter thru a nitrite-free filter. Return the first runnings to the filter until a clear filtrate is obtained. Pipet 50 cc of the filtrate and 50 cc of the standard nitrite soln into small flasks; add to each 50 cc of $H_2O$ and 2 cc each of the sulfanilic acid and alpha-naphthylamine hydrochloride solns; shake; and allow to stand 1 hour to bring out the color. Compare the two solns in a colorimeter. Divide the height of the column of the standard soln by that of the soln of the sample to obtain the parts of nitrous N (free and combined) per million of flour.

### 45 GASOLINE COLOR VALUE—TENTATIVE

Place 20 g of the flour in a wide-mouthed, glass-stoppered 120 cc bottle and add 100 cc of colorless gasoline. Stopper tightly and shake vigorously for 5 min. After standing 16 hours, shake again for a few seconds until the flour has been loosened from the bottom of the bottle and thoroly mixed with the gasoline; then filter immediately thru a dry 11 cm paper into an Erlenmeyer flask, keeping the funnel covered with a watch-glass to prevent evaporation. In order to secure a clear filtrate, allow a certain quantity of the flour to pass over into the filter, and pass the first portion of the filtrate thru a second time. It will be found convenient to fit the filter paper to the funnel by means of $H_2O$ and to dry thoroly either by standing overnight in a well-ventilated place or by heating.

Determine the color value of the clear gasoline soln in a Schreiner or similar colorimeter, using for comparison a 0.005% $K_2CrO_4$ soln. This soln corresponds to a gasoline number of 1.0 and is conveniently prepared by diluting 10 cc of a 0.5% soln to

1 liter. The colorimeter tube, containing the gasoline soln, should first be adjusted so as to read 50 mm; then the tube containing the standard chromate soln should be raised or lowered until the shades of yellow in both tubes match. The reading of the chromate soln, divided by the reading of the gasoline soln, gives the gasoline color value. The color value may be determined also in Nessler tubes, using for comparison $K_2CrO_4$ solns of various dilutions prepared from a 0.5% soln and filling the tubes in all cases to the height of 50 mm.

## BAKED CEREAL PRODUCTS

### BREAD

### 46 PREPARATION OF SAMPLE[19]—TENTATIVE

(To be used when total solids of original entire loaf is not desired)

Cut the loaf or ½ the loaf of bread into slices 2–3 mm thick. Spread the slices on paper and allow them to dry in a warm room until sufficiently crisp and brittle to grind well in a mill. Grind the entire sample to pass a 20-mesh sieve, mix well, and keep in an air-tight container.

### 47 TOTAL SOLIDS IN AN ENTIRE LOAF OF BREAD[20]—OFFICIAL

Accurately weigh the loaf of bread immediately upon receipt (*A*). Use scales sensitive to at least 0.2 g. (When determining whether bread is in conformity with the Department of Agriculture standards do not weigh the loaf sooner than 1 hour after removal from the oven.) Should accurate weighing be impossible at this time, seal the sample in an air-tight container and accurately weigh as soon thereafter as is practicable (*A*). Preserve the sample in such a manner that no loss of bread solids can occur, whereby the loss would be calculated as moisture. Cut the bread into slices 2–3 mm thick (½ of the loaf may be used—official, first action). Spread the slices on paper; allow them to dry in a warm room (approximately 15–20 hours); and when apparently dry, break into fragments. If the bread is not entirely crisp and brittle, allow it to dry longer—until it is in equilibrium with the moisture of the air—in order that no moisture changes may occur during grinding. Quantitatively transfer the air-dried bread to the scale pan and accurately weigh (*B*). Grind the sample just to pass a 20-mesh sieve, mix well, and keep in an air-tight container. Determine the percentage of total solids (*C*) of the ground sample as directed under **3** or **5**. Calculate total solids of the bread from the formula—

$$T.S. = \frac{\frac{B \times C}{100} \times 100}{A}, \text{ or } \frac{B \times C}{A}, \text{ in which}$$

$A$ = weight of loaf (or ½ loaf) at time of receipt,
$B$ = weight of the air-dried sliced bread; and
$C$ = percentage of total solids in the prepared ground sample.

### TOTAL SOLIDS OF AIR-DRIED GROUND SAMPLE

### 48 *Method I—Tentative*

Use 2 g of sample prepared as directed under **46** and proceed as directed under **3**.

### 49 *Method II—Official, first action*

Proceed as directed under **5**.

### 50 ASH—OFFICIAL[21]

Use 3–5 g of sample prepared as directed under **46** and proceed as directed under **6**.

### 51 CHLORIDES IN ASH—OFFICIAL, FIRST ACTION

Proceed as directed under **64**.

### 52 PROTEIN—OFFICIAL

(Organic and Ammoniacal Nitrogen)

Determine N as directed under **II, 19, 22** or **24**, using 2 g of air-dried ground sample prepared as directed under **46**. Multiply the percentage of N by the factor 5.7 to obtain the percentage of protein.

## FAT

### 53 *Method I[22]—Official, first action*

Place 5 g of the ground sample in a 200 cc Erlenmeyer flask and add a mixture of 10 cc of 95% alcohol, 2 cc of $NH_4OH$, and 3 cc of $H_2O$. Place the flask on a steam bath and maintain the contents at the boiling point for 2 min. Cool, and extract the mixture with 3 successive 25 cc portions of ether, kneading and tamping the matted material thoroly each time with a glass rod flattened at the end. Pour off the ether layer by decantation into a 250 cc beaker, draining off the last 25 cc portion of ether as completely as possible. Add another 15 cc portion of the ammoniacal alcoholic soln to the extracted residue in the flask and disintegrate the matted material as thoroly as possible by means of the flattened glass rod, which should be left in the flask for that purpose. Return the flask to the steam bath as before, taking care that no loss of material occurs thru bumping, due to the presence of ether. Boil 2 min. Again extract with 3 successive 25 cc portions of ether, adding the ether extracts to those obtained in the first extraction. Evaporate the combined extracts to dryness on a steam bath and then extract the fatty residue with 5 or 6 successive 15 cc portions of a mixture of equal volumes of ether and petroleum ether. Collect the extracts in a weighed dish (do not try to filter) and evaporate to dryness on a steam bath. Dry the residue to constant weight in an oven at the temp. of boiling $H_2O$, cool in a desiccator, and weigh.

### 54 *Method II—Acid hydrolysis method—Official, first action*

Proceed as directed under **8**.

### 55 CRUDE FIBER—OFFICIAL, FIRST ACTION

Proceed as directed under **XXVII, 17–19**.

## EXPERIMENTAL BAKING TEST[23]—TENTATIVE

*Basic Procedure*

### 56 EQUIPMENT

*Mixing bowl.*—Ordinary graniteware "oatmeal bowl," top diameter 16.5 cm, bottom diameter 5.7 cm, and depth 7.3 cm.

*Fermentation bowl.*—Same as mixing bowl, but smaller. Top diameter 12.7 cm, bottom diameter 5 cm, and depth 5.7 cm.

*Spatula.*—Flexible steel blade, approximately 12.7 cm long by 1.9 cm wide.

*Baking pan.*—Dimensions: Bottom inside, 53 x 93 mm; height (sides), 85 mm; height (ends) 68 mm; top inside (at height of 68 mm) 60 x 105 mm. Pans to be made from 4X (0.55 mm) spotless metal, which requires no greasing.

*Fermentation cabinet and oven*—With precise temperature control.

*Volume measuring apparatus.*—Calibrate gravimetrically.[24] It is suggested that four points of known volumes, namely, 300, 400, 500, and 600 cc, be determined. By plotting these four points against their corresponding values, as measured by the apparatus, the calibration curve for the apparatus is obtained. This calibration likewise corrects for peculiarities of the operator, providing such are uniformly and consistently practised.

*Thermometers:*

*A. For fermentation cabinet and dough testing.*—Use a dough testing thermometer graduated from 15 to 40° or its equivalent on the Fahrenheit scale.

*B. For oven.*—Use an oven thermometer that has a range from 100 to 260° or its equivalent on the Fahrenheit scale.

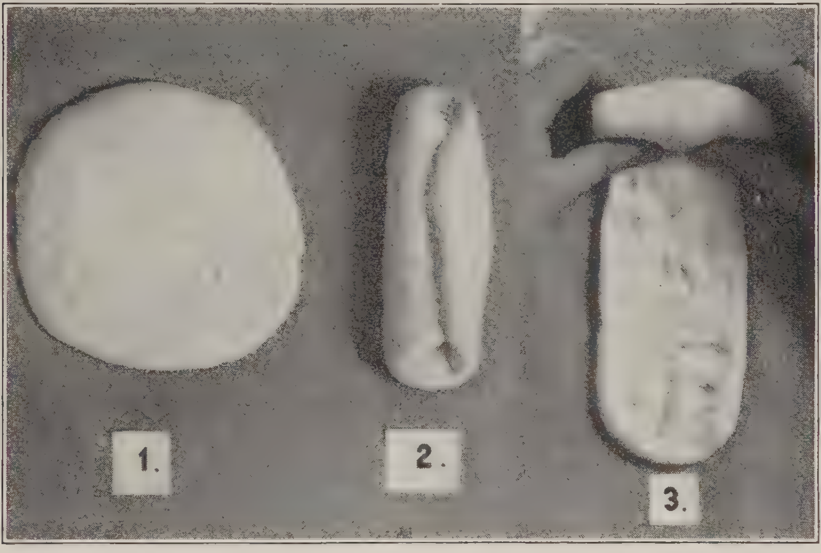

FIG. 19.—PREPARING THE DOUGH FOR PANNING

### 57 INGREDIENTS

*Flour.*—Weight equivalent to 85 g of dry flour, or 100 g of flour on a 15% moisture basis.

*Yeast.*—3 g.

*Salt.*—1 g.

*Sugar* (sucrose).—2.5 g.

*Water* (distilled).—Sufficient to make 58% absorption with the flour on a 15% moisture basis. To determine the exact quantity of $H_2O$ to be added subtract from 158 the weight of flour used as previously computed.

### 58 PROCEDURE

*Mixing.*—Dissolve the salt and sugar in a portion of the $H_2O$. Disintegrate the weighed portion of yeast in the salt-sugar soln or in another portion of the $H_2O$. If the former procedure is followed, do not allow the yeast to remain in the salt-sugar soln for any considerable time before adding to the flour. If stock solns are used, correct for the $H_2O$ added. Add the flour and mix with a flexible spatula that will conform readily to the shape of the bowl, making 125 cuts with the spatula. So regulate the temp. of the ingredients that the dough comes from the mixing operation at 30°. Remove the dough from the bowl and fold 20 times in the hands.

*Fermentation.*—Place the dough in a fermentation bowl and allow to ferment for 105 min. at 30° (plus or minus 0.5°) and at not less than 75% relative humidity. Remove the dough from the bowl, fold 15 times in the hands (first punch), return the dough to the bowl, and allow fermentation to proceed as before for 50 min. Again remove the dough, fold 10 times (second punch), and replace in the fermentation bowl for 25 min. Remove the dough from the bowl, mold, and pan as follows:

*Molding and panning.*—Place the dough on a table or molding board and pound vigorously with the heel of the hand until the dough is flat and circular (Fig. 1). Holding one side of the dough, cut the mass loose from the table with the spatula, and turn on the reverse side. Fold over two opposite sides so that they overlap to a considerable degree (Fig. 2). Turn the dough over, and again pound it flat with the heel of the hand. Holding one end, cut the dough loose from the table with a spatula, and turn on reverse side with the seam of the dough running from the operator. Starting at the more remote end, roll the dough toward the operator, folding it as tightly as possible (Fig. 3). Seal the seam tightly, and with the seam underneath seal the ends by pinching them vertically. Roll lightly under the palm of the hand, adjusting the dough to the length of the pan, and place in the pan with the seam down. (The length of the dough prior to the final light rolling should not exceed that of the pan. Use no dusting flour in the molding process.

*Proof.*—Proof 55 min. under the same conditions as for fermentation.

*Baking.*—Bake 25 min. at a temp. of 230° (plus or minus 5°) at the level of the top of the baking pan. (Precise control of temp., both as to degree and uniformity, is essential.)

*Measurement.*—Weigh the loaf and measure its volume 30 min. after removal from the oven.

### 59 *Optional Variations*

1. *Absorption:* Basic procedure in which absorption only is varied.
2. *Fermentation:* Basic procedure varying fermentation time only.
3. *Addition of special oxidizing reagent $KBrO_3$*: Basic procedure with addition of $KBrO_3$ in successive increments of 1 mg. Prepare a soln of $KBrO_3$ so that 1 cc = 1 mg of $KBrO_3$, using $KBrO_3$ of highest purity.
4. *Method of mixing:* Basic procedure varying mixing by use of mechanical mixer.

## ALIMENTARY PASTES

### 60 COLLECTION AND PREPARATION OF SAMPLE[25]—TENTATIVE

Select from the lot to be analyzed sufficient strips or pieces to assure a representative sample. Break these into small fragments with the hands or in a mill and mix well. Grind 300–500 g in a mill until all the material just passes thru a 20-mesh sieve. Keep the ground sample in a sealed container to prevent moisture changes.

### TOTAL SOLIDS AND MOISTURE —OFFICIAL, FIRST ACTION

### 61 *I. Indirect Method*[26]

Determine the total solids in the sample, prepared as directed under **60**, as directed under **3**.

### 62 *II. Air Oven Method*

Proceed as directed under **5**, using a sample prepared as directed under **60**.

### 63 ASH—OFFICIAL

Proceed as directed under **6**, using 3–5 g of the sample prepared as directed under **60**.

### 64 CHLORIDES IN ASH AS SODIUM CHLORIDE—OFFICIAL

Dissolve the ash obtained under **63** in $HNO_3$ (1+9), filter, wash the filter paper with hot $H_2O$, and determine Cl in the combined filtrate and washings as directed under **XII, 33** or **35**. Calculate the Cl to its equivalent of NaCl.

### 65 FAT (ACID HYDROLYSIS METHOD)[27]—OFFICIAL, FIRST ACTION

Place 2 g of the sample in a Röhrig or Mojonnier fat extraction tube, add 2 cc of 95% (by volume) alcohol, and shake so as to moisten all particles. (The moistening of the sample with alcohol prevents lumping on addition of the acid.) Add 10 cc of HCl (25+11), mix well, set the tube in a water bath held at 70–80°, and shake at frequent intervals for 30–40 min. Fill to within 1–2 cc of the mark with 95% alcohol and cool. Add 25 cc of ethyl ether and shake the mixture well. Then add 25 cc of redistilled petroleum ether (b. p. below 60°) and mix well. Let stand until the upper liquid is practically clear and proceed as directed under **8**, beginning "Draw off as much as possible."

### 66 CRUDE FIBER—OFFICIAL, FIRST ACTION

Proceed as directed under **XXVII, 17–19**.

### 67 PROTEIN—OFFICIAL

(Organic and Ammoniacal Nitrogen)[28]

Determine the N as directed under **II, 19, 22,** or **24,** using 1 g of the sample prepared as directed under **60**. Multiply the percentage of organic and ammoniacal N by the factor 5.7 to obtain the percentage of protein.

### 68 WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL—OFFICIAL, FIRST ACTION

Proceed as directed under **25**.

### 69 LIPOID AND LIPOID PHOSPHORIC ACID ($P_2O_5$)—OFFICIAL, FIRST ACTION

Proceed as directed under **26** and **27**.

### 70 UNSAPONIFIABLE RESIDUE—TENTATIVE

*I. F.A.C. Method*

Proceed as directed under **28**, using 5 g of alimentary paste.

### 71 *II. Kerr-Sorber Method*

Proceed as directed under **33**.

### 72 EXTRACTION AND IDENTIFICATION OF ADDED COLOR[29]—OFFICIAL

Transfer 50 g of the sample prepared as directed under **60** to a 200 cc nursing bottle; add about 125 cc of amyl alcohol; stopper; and shake well. Add 27 cc of HCl (1+1) and agitate in a mechanical shaker until most of the color is extracted (15–30 min.). Centrifugalize and pour off the amyl alcohol into a 250 cc separatory funnel. Separate the colors in the amyl alcohol fractionally by successive washings with

HCl of decreasing concentrations, as 4 *N*, *N*, 0.25 *N*, etc., and then with $H_2O$ until the washings are neutral (**XXI, 7**). (A roughly approximate 4 *N* acid is made by diluting 300 cc of HCl to 1 liter, and the other concentrations by using 75 cc, 19 cc, etc., of strong acid, respectively.) Now add an equal volume of petroleum ether (b. p. below 60°) to the amyl alcohol and wash again with $H_2O$. Finally wash the ether-alcohol mixture with 10% NaOH soln. Most of the egg and wheat colors as well as basic coal tar dyes and some others remain in the amyl alcohol-ether mixture. Identify the colors in the various fractions as directed under **XXI, 12** and **19.** Some colors are more or less colorless in acid soln and are apparent to the eye only after neutralization.

### 73 DETECTION OF THE PRESENCE OF WHOLE EGG OR COMMERCIAL YOLK SOLIDS—TENTATIVE

Calculate the following ratios as percentages:

(1) $\frac{100W}{N}$, (2) $\frac{100W}{L}$, and (3) $\frac{100P}{W}$, in which

W = the water-soluble protein-N precipitable by 40% alcohol (**24**);
*N* = the organic and ammoniacal N (**67**)
*L* = the lipoids (**26**); and
*P* = the lipoid $P_2O_5$ (**27**).

Compare the values of these ratios with those obtained in the analysis of noodles of known composition.[30]

### 74 EGG SOLIDS[29]—TENTATIVE

Calculate the percentage of egg solids in the sample from the lipoid $P_2O_5$ content by means of the following basic values and formulas.

(**a**) Basic values:

0.055% = lipoid $P_2O_5$ of flours, average value (dry basis);
1.38 % = lipoid $P_2O_5$ of whole eggs, average value (dry basis); and
1.78 % = lipoid $P_2O_5$ of commercial yolk, average value (dry basis).

(The above basic values are from a limited number of analyses, but it is extremely unlikely that more extended investigations will alter them materially.)

(**b**) Formula for percentage of whole egg solids:

$$E = \frac{(A - 0.055)\ 100}{1.38 - 0.055} \text{ or } (A - 0.055)\ 75.5, \text{ in which}$$

*E* = percentage of whole egg solids in sample (dry basis); and
*A* = percentage of lipoid $P_2O_5$ in sample (dry basis) times 1.1, to correct for loss of lipoid $P_2O_5$ in manufacturing process.[31]

Then

$$\text{Whole egg solids in original sample} = \frac{E \times \text{percentage of dry matter in noodles}}{100}$$

(**c**) Formula for percentage of commercial egg yolk:

For samples made with commercial egg yolk use the following formula:
$Y = (A - 0.055)\ 58.0$, in which
*Y* = percentage of commercial yolk solids in sample (dry basis); and
*A* = percentage of lipoid $P_2O_5$ in sample (dry basis) times 1.1 to correct for loss of lipoid $P_2O_5$ in manufacturing process.[31]

Then

$$\text{Commercial yolk solids in original sample} = \frac{Y \times \text{percentage of dry matter of noodles}}{100}$$

## SELECTED REFERENCES

[1] J. Assoc. Official Agr. Chem., **9,** 39 (1926).
[2] Ibid., **8,** 665 (1925); **9,** 39 (1926).
[3] Ibid., **8,** 665 (1925); **9,** 40 (1926).
[4] Ibid., **7,** 132 (1923).
[5] Ibid., **6,** 508 (1922); **9,** 41 (1926).
[6] Ibid., **10,** 33 (1927); **11,** 37 (1928).
[7] U. S. Dept. Agr. Bur. Chem. Bull., **152,** p. 104.
[8] Ibid., **122,** p. 54.
[9] J. Assoc. Official Agr. Chem., **12,** 39 (1929).
[10] Ann. Phys. Chem., **85,** 161 (1852); U. S. Dept. Agr. Bur. Chem. Bull. **122,** p. 127.
[11] J. Assoc. Official Agr. Chem., **7,** 84 (1923); **12,** 40 (1929); **14,** (1931).
[12] Ibid., **7,** 91 (1923); **9,** 40 (1926).
[13] Ibid., **7,** 91 (1923); **9,** 41 (1926).
[14] Ibid., **9,** 45 (1926); **10,** 35 (1927); **11,** 37 (1928).
[15] Ibid., **8,** 441 (1925); **10,** 33 (1927).
[16] Ibid., **10,** 108 (1927); **11,** 37 (1928).
[17] Ibid., **6,** 68 (1922); **7,** 130 (1923).
[18] Ibid., **11,** 134 (1928).
[19] Ibid., **9,** 42 (1926).
[20] Ibid., **8,** 585 (1925); **9,** 42 (1926).
[21] Ibid., **9,** 42 (1926).
[22] Ibid., **6,** 61 (1922).
[23] Ibid., **12,** 41 (1929).
[24] Cereal Chem., **5,** 220 (1928).
[25] J. Assoc. Official Agr. Chem., **9,** 43, 396 (1926).
[26] Ibid., **9,** 43, 397 (1926).
[27] Ibid., **11,** 38 (1928); **6,** 508 (1923).
[28] Ibid., **9,** 43 (1926).
[29] Ibid., **6,** 12 (1922); **8,** 109(1924); U. S. Dept. Agr. Bull. **448.**
[30] J. Assoc. Official Agr. Chem., **7,** 84 (1923); 407 (1924); **8,** 435 (1925).
[31] Ibid., **7,** 91 (1923).

# XXI. COLORING MATTERS IN FOODS—TENTATIVE

(The numbers in parentheses and brackets following the name of a dye represent in the first instance the number of that dye as listed in "A Systematic Survey of the Organic Colouring Matters," founded on the German of Drs. G. Schultz and P. Julius, 1904, by Arthur G. Green, while the second number designates the number as listed in the Society of Dyers and Colourists' "Colour Index," first edition, January, 1924.)

## 1 PIGMENTS

Separate the insoluble pigments, ultramarine, lampblack, etc., which are most commonly used as facings, by washing the sample with $H_2O$ and allowing the washings to settle. Identify the particles of coloring matter by microscopical examination and treat the residue or purified coloring matter with chemical reagents. A large proportion of the common pigments other than lakes, such as the yellow, brown, and red ochres and umbers, are derivatives of the heavy metals and contain Fe, Mn, etc. Others, such as the green and blue compounds, including certain green chlorophyll derivatives, may contain Cu. These pigments may be identified by the usual tests for the respective metals. The analytical properties of the insoluble coloring matters are described in various standard works, some of which are listed under the selected references, especially Farbstofftabellen by Schultz[1] and Colour Index.

## SOLUBLE COLORING MATTERS AND THEIR LAKES

### WOOL DYEING PROCEDURE[2]

### 2 *I. Water-soluble coal tar dyes*

(a) *Wines fruit juices, distilled liquors, flavoring extracts, vinegars, beers, sirups, non-alcoholic beverages, and similar products.*—Dilute 20–200 cc of the sample with 1–3 volumes of $H_2O$, neutralize with $NH_4OH$ (1+9) if necessary, and boil or heat on a steam bath with a small piece of white woolen cloth (nun's veiling). If the mixture contains much alcohol, heat until most of it has been removed; in other cases take out the wool after 5–15 min. and rinse with $H_2O$. Then treat the liquid with 3 or 4 drops of HCl for each 100 cc of soln and warm again for 10–20 min. with a clean piece of wool. If the wool takes up much coloring matter in either case, the presence of coal tar dyes is indicated.

The basic colors dye the fiber best from neutral or faintly ammoniacal solns and, if present, they will appear on the first piece of wool. Acid colors dye from neutral solns, but more readily from those containing free acid. The lichen colors[3] (archil, cudbear, litmus) go readily on wool, however, and many other natural colors, such as turmeric, will dye the fiber if present in considerable amount. On the other hand, a few coal tar dyes, especially auramine O and naphthol green B, are quite unstable, and if present in small quantities may give no distinct dyeing. Acid dyes are much more frequently used than basic dyes, and in most cases they may be removed from wool without much decomposition by "stripping" the latter with dilute $NH_4OH$.[4] Many natural colors are destroyed by the action of the alkali, while others remain for the most part on the fiber.

If the behavior with wool in neutral and acid solns indicates the presence of acid dyes, rinse the colored cloth thoroly with $H_2O$, cover with $NH_4OH$ (1+9) in a casserole, and boil for a few min. Remove the cloth and squeeze out the adhering liquid. Boil the ammoniacal soln to remove the excess of $NH_3$, drop in a piece of clean wet wool, make distinctly but not strongly acid with HCl (1+9), and boil again. If acid coal tar dyes are present, they will usually give a fairly clean, bright dyeing on the second piece of wool. A further purification may be carried out by repeating the stripping and redyeing, tho this procedure is generally accompanied by a corresponding loss of dye.

(b) *Candies and similar colored sugar products.*—Dissolve about 20 g of the sample in 100 cc of $H_2O$ and treat the soln as directed under (a). When the coloring matter is on the surface of the candy, pour off the soln before the colorless inner portion has dissolved.

(c) *Jams and jellies.*—Boil a mixture of 10–20 g of the sample and 100 cc of $H_2O$ with wool in neutral and also in acid soln as directed under (a). For thick jams it is usually better, tho less easy, first to extract the coloring substances by treating the product as directed under (d).

(d) *Canned and preserved fruits and vegetables, sausage casings, smoked fish, coffee, spices, etc.*—Macerate 20–200 g of the sample with 4–5 times its weight of alcohol, 80% by volume. Allow to stand a few hours, pour off the solvent as completely as possible, and repeat the extraction, using alcohol 70% by volume and containing approximately 1% of $NH_4OH$. (1) Examine separately the filtered alcoholic extracts as directed under (a); or, (2) boil the ammoniacal soln until practically neutral, complete the neutralization with acetic acid, add the neutral 80% alcohol extract, continue the evaporation until most of the alcohol is removed, and boil a small portion with wool as directed under (a).

(e) *Cocoa and chocolate products.*—Treat cocoa as directed under (d). The alcoholic extract will contain large quantities of natural coloring matters, and several dyeings and strippings may be necessary to remove these in order to show the presence of coal tar dyes.

Chocolate may be treated similarly, but the following procedure is preferable: Wash 20–200 g of the well-divided sample with gasoline on a filter until most of the fat has been removed; if the gasoline is colored, reserve for the examination of oil-soluble dyes as directed under **3**. Remove most of the adherent solvent from the residue by evaporation or pressure between layers of absorbent paper and digest with alcohol as directed under (d).

Coal tar dyes may also be detected in chocolate and cocoa products by mixing the samples directly with 3–4 times their weight of hot $H_2O$ and immediately boiling the magma with wool, as directed under (a).

(f) *Cereal products.*—Proceed as directed under (d), in most cases working with a large quantity of the sample, 200–500 g, and a relatively smaller quantity of alcohol. If tests are to be made for the acid dyes, only the extraction with neutral 80% alcohol may be omitted advantageously. In the case of macaroni products, proceed as directed under **XX, 72.**

### 3 *II. Oil-soluble coal tar dyes*[5]

Prepare an alcoholic soln of the dye by applying one of the following methods to the oil or fat obtained by extraction with ether or gasoline, if the nature of the substance requires it:

(a) Shake the oil or melted fat with an equal volume of alcohol, 90% by volume, and wash the alcoholic extract with several portions of gasoline to free the coloring matter from foreign fats. The alcohol, after separation, will contain aniline yellow, butter yellow, aminoazotoluene, auramine, etc., if present.

(**b**) Saponify 20–200 g of the oil or fat with 0.5 *N* alcoholic KOH, remove most of the alcohol on the steam bath, and extract the soap with ether or gasoline. Remove the dyes from the solvent with 10 cc portions of HCl (1+3). Most of the common dyes are removed by this treatment, tho the digestion with strong alkali may cause some decomposition and make the extraction rather troublesome.

(**c**) Dilute 20–200 g of the oil or melted fat with 1–2 volumes of gasoline and shake out successively with 2–4% KOH or NaOH soln, HCl (1+3), and $H_3PO_4$-$H_2SO_4$ mixture, prepared by mixing 85% $H_3PO_4$ with about 10–20% by volume of $H_2SO_4$. The dilute alkali extracts sudan G (10) [23] and annatto (709) [1241]. The dilute HCl extracts aniline yellow (7) [15], aminoazotoluene (—) [17], and butter yellow (16) [19], the first two forming orange-red, the latter cherry-red solns in this solvent. The $H_3PO_4$ mixture is necessary for the extraction of sudan I (11) [24], sudan II (49) [73], sudan III (143) [248], and the homologue of the last, sudan IV (—) [258]. Benzeneazo-beta-naphthylamine (—) [22] and homologues also come in in this group, tho they readily undergo chemical changes in the strongly acid mixtures. The procedure is not very suitable in the presence of auramine, but this dye is seldom found in oils. Neutralize the alkaline and the dilute HCl solns; dilute the $H_3PO_4$ mixture and partially neutralize, cooling the liquid during this operation; and extract the dyes by shaking with ether or gasoline.

For the direct dyeing test use the alcoholic soln obtained as directed under (**a**). Evaporate to dryness the ether or gasoline solns obtained as directed under (**b**) and (**c**) and dissolve the residue in 10–20 cc of 95% alcohol. To the alcoholic soln add some strands of white silk and a little $H_2O$ and evaporate on a steam bath until the alcohol has been removed or the dye is taken up by the silk. The dyeing test is sometimes unsatisfactory, and in all cases a small portion of the alcoholic soln should be tested by treating with an equal volume of HCl and $SnCl_2$ soln. The common oil-soluble coal tar dyes are rendered more red or blue by the acid and are decolorized by the reducing agent. Most of the natural coloring matters become slightly paler with the acid and are little changed by the $SnCl_2$ soln.

## SEPARATION OF COLORING MATTERS IN PURE CONDITION BY MEANS OF IMMISCIBLE SOLVENTS[6]

### 4 *Coal Tar Dyes in General*

The use of immiscible solvents for the separation of mixtures of coloring matters usually requires a systematic fractionation since many dyes do not differ very greatly in their solubilities in the various solvents.

### 5 PREPARATION OF SOLUTION

(**a**) *Water-soluble colors.*—Proceed as directed under **2**, omitting the fixation of the color on wool, and obtain an aqueous soln as free as practicable from suspended matter, alcohol, acids, alkalies, and salts. Liquids require no preparation except the removal of any alcohol that may be present.

(**b**) *Water-insoluble lakes.*—If the sample is in solid form, treat the well-divided material with sufficient $H_2O$ to form a paste.

(**c**) *Oil-soluble dyes.*—Proceed as directed under **3**, preferably **3** (**a**) or **3** (**c**).

The dye concentration should lie preferably between 0.01 and 0.05%. The soln obtained in the examination of colored food products rarely requires further dilution, but with commercial food colors the concentration should not be too great.

### 6 *Basic Dyes*

Most basic dyes may be separated from mixtures by making alkaline with 10% NaOH soln and shaking with ether.[7] Use the sample, prepared as directed under **5**, for this purpose. Separate the ether layer, which may or may not be colored; wash it twice with a few cc of $H_2O$ to remove excess of alkali; and shake with acetic acid (1+18), which will take up any dye present and form a colored soln. Altho this treatment may, to some extent, alter the common basic colors, it can be used for the detection of methyl violet B (451) [680], magenta (448) [677], bismarck brown (197) [331], malachite green (427) [657], and rhodamine B (504) [749]. With care auramine (425) [655] also may be separated in this way, tho it is quickly decomposed on standing in alkaline soln.

### 7 *Acid Dyes*

The following short procedure is often convenient for the examination of mixtures of acid dyes: Make the sample, prepared as directed under **5**, strongly acid by adding $\frac{1}{2}$ its volume of HCl and shake with amyl alcohol. Separate the amyl alcohol soln and wash by shaking with successive portions of $\frac{1}{2}$ its volume of $H_2O$, reserving the portions in separate test tubes or beakers. Because of the varying acid content of the amyl alcohol these washings will show a regular decrease in acidity, and the coloring matters will appear in maximum quantity in the different fractions according to their respective solubilities. Ponceau 6R (108) [186] is washed out chiefly while the acidity is still high, normal or above. Amaranth (107) [184), brilliant scarlet (106) [185], and tartrazine (94) [640] appear when the washings have an acidity between normal and 0.25 $N$; orange G (14) [27] and soluble blue (480) [707] between 0.5 $N$ and $\frac{1}{16}$ $N$; and palatine scarlet (53) [77], ponceau 2R (55) [79] and 3R (56) [80], naphthol yellow S (4) [10], cochineal (706) [1239], crystal ponceau (64) [89], and azorubine A (103) [179] between $\frac{1}{16}$ $N$ and 1/256 $N$. When practically all the acid is removed, orange I (85) [150], orange II (86) [151], and croceine orange (13) [26] begin to wash out, and less readily, orange IV (88) [143] and metanil yellow (95) [138]. Finally the unsulfonated coloring matters, such as erythrosine G (516) [772], erythrosine B (517) [773], and the rose bengals (520) [777] and (523) [779] are removed very slowly by $H_2O$ or not at all unless the solvent is diluted with gasoline and the dyes are removed with $H_2O$ containing a few drops of $NH_4OH$. Acid yellow (8) [16] and brilliant yellow S (89) [144] are not very uniform in composition. They are partially taken up by amyl alcohol from acid soln and appear chiefly in the first washings. Indigotine (692) [1180] behaves somewhat similarly.

When it appears probable that only the coal tar dyes listed in the regulations for the enforcement of the Federal Food and Drugs Act[8] for use in food products are present, the following abridged procedure may be conveniently used for their separation:

## 8 PERMITTED COAL TAR FOOD COLORS[8]

(*Amaranth, ponceau 3R, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, indigotine, naphthol yellow S, tartrazine, yellow AB, and yellow OB.*) No methods have been adopted for brilliant blue, ponceau SX, and sunset yellow.

### PREPARATION OF SOLUTION

**(a)** *For foodstuffs containing oil-soluble dyes.*—Proceed as directed under **3** **(a)**, evaporate the 90% alcoholic extract to dryness in a casserole, treat the residue with 40 cc of low boiling gasoline, and shake the gasoline soln with 2 or 3 portions of 5 cc each of 2–4% NaOH soln (to remove annatto, turmeric, etc., if present). The gasoline soln will contain the yellow OB and yellow AB.

**(b)** *For foodstuffs which contain no oil-soluble dyes or from which these dyes have been removed.*—Proceed as directed under **2**, omitting the fixation of the color on wool and obtain an aqueous soln as free as possible from suspended matter, alcohol, acids, alkalies, and salts. The dye soln should be preferably between 0.01 and 0.05%. The soln obtained in the examination of colored food products rarely requires further dilution, but with commercial food colors care must be taken that the concentration is not too great.

### 9 SEPARATION

(a) *Yellow AB and yellow OB.*—Extract the gasoline soln of these dyes, as obtained under **8** (a), 3 times with ½ its volume of 13 *N* $H_2SO_4$. Shake each acid extract successively with 2 portions (equal volumes) of low-boiling gasoline, using the same 2 portions of gasoline for each acid portion. Extract each of the 2 latter gasoline portions with 20 cc of 13 *N* $H_2SO_4$, using the same acid portion successively for both gasoline portions. Finally extract the second of these gasoline portions with another 20 cc portion of 13 *N* $H_2SO_4$. (The original gasoline soln has now been shaken with acid 3 times, the next gasoline portion 4 times and the third 5 times.) Combine the acid extracts, dilute with water, re-extract with low-boiling gasoline, and evaporate the solvent. Yellow AB will be found in a practically pure state. Combine the gasoline solns(original and subsequent solns left after the acid washings), wash with small portions of $H_2O$ to remove excess of acid, and evaporate the solvent. The yellow OB will remain as a residue. (This method is not absolutely quantitative, but it is sufficiently accurate to make a separation of either of the dyes with comparatively little contamination from the other.) The following color test may be applied to the separated dyes to confirm their identity: Shake 5 cc of a neutral gasoline soln of the dye in a test tube with 5 cc of a mixture of 1 part of 40% HCHO soln and 4 parts of acetic anhydride. Both coloring matters are extracted by the acetic anhydride, yellow AB giving in a few seconds a red colored soln and yellow OB, under the same conditions, giving an orange colored soln.

(b) *Amaranth, ponceau 3R, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, indigotine, naphthol yellow S, and tartrazine.*—To the soln obtained under **8** (b), add sufficient 25% salt soln to make the concentration about 10% and 1 part acetic acid to every 7 parts of soln. Extract with 3–50 cc portions of amyl alcohol. Draw off the lower layer and reserve for further treatment. Wash the amyl alcohol extract in rotation with 25 cc portions of 5% salt soln until the washings are colorless or nearly so. Add the washings to the original aqueous soln. Dilute the amyl alcohol extract with equal volume of gasoline and wash with 25 cc portions of $H_2O$ until all color is extracted. The coloring matters obtained are orange I and guinea green B. For their separation see (1) below. Treat the amyl alcohol gasoline soln with 10 cc portions 0.1 *N* NaOH or with 10 cc portions of $NH_4OH$ (1+9), which will remove erythrosine. The original soln and washings (from which the 3 named dyes were removed) are acidified with HCl (1 volume acid to 40 volumes of soln) and extracted in 50 cc volumes with 3–50 cc portions of amyl alcohol. Reserve the lower aqueous layer for further treatment. Wash amyl alcohol extract with 25 cc portions of 0.25 *N* HCl until the washings are colorless or nearly so. Combine washings with the aqueous soln of above. The amyl alcohol is extracted with several 25 cc portions of $H_2O$ until all color is extracted. The coloring matters obtained are ponceau 3R and naphthol yellow S. For their separation see (2). The original soln and washings (from which the 5 named dyes were removed) are treated in 50 cc volume with 3–50 cc portions of dichlorhydrin. Reserve the upper aqueous layer for further treatment. Wash the dichlorhydrin extract in rotation with several 20 cc portions of 25% salt soln. Combine washings with the aqueous soln of above. The dichlorhydrin extract is diluted with 2 volumes of $CCl_4$ and extracted with several 25 portions of $H_2O$ until all color is extracted. The coloring matters obtained are light green SF yellowish and fast green FCF. For their separation see (3). Further acidify the original soln and washings (from which the 7 named dyes were removed) with HCl (1 volume of acid to 40 volumes of soln) and extract in 50 cc volumes with 3–50 cc portions of amyl alcohol. If the color intensity of the soln was not too strong, all coloring matter should have been extracted by the solvent. Discard the lower colorless or nearly colorless layer and wash out the dyes from the amyl alcohol extract in rotation with several 25 cc portions of $H_2O$, until all color is extracted. The coloring matters obtained are indigotine, amaranth and tartrazine. For their separation see (4).

(1) *Orange I and guinea green B.*—Extract the combined colors with 2–20 cc portions of $\alpha$ dichlorhydrin. Discard the colorless upper aqueous layer, dilute the solvent with 2 volumes of $CCl_4$, and extract out orange I in rotation with several 10 cc portions of $H_2O$, and guinea green B with several 10 cc portions of 25% alcohol.

(2) *Ponceau 3R and naphthol yellow S.*—Acidify the combined colors with HCl (1 part acid to 10 parts of soln) and extract the naphthol yellow S with 2-20 cc portions of washed ethyl acetate or amyl acetate. Ponceau 3R is not extracted appreciably and remains in the aqueous layer. Wash the solvent with 5 cc portions of *N* HCl to remove traces of ponceau 3R. Naphthol Yellow S is removed from the combined ethyl acetate or amyl acetate with 5 cc portions of $NH_4OH$ (1+9).

(3) *Light green SF yellowish, and fast green FCF.*—The combined colors are treated with an equal volume of 2 *N* $Na_2CO_3$ soln and extracted in 25 cc volumes with 2–50 cc portions of *N* butyl alcohol. Draw off the lower aqueous layer containing the fast green FCF and remove the last traces with 25 cc portions of *N* $Na_2CO_3$ until the washings are colorless. The light green SF yellowish can be removed from the butyl alcohol with 10 cc portions of 1% acetic acid.

(4) *Indigotine, amaranth and tartrazine.*—to separate the indigotine heat a small portion of the soln, which should be neutral or faintly acid, to boiling, and add a few crystals of $NaHSO_2$ until all the dyes are reduced. On shaking with air the indigotine is quickly restored, while amaranth and tartrazine are destroyed. To the remainder of the mixed dye soln add several decigrams of urea, heat, and while the mixture is boiling add 1 or 2 drops of 10% $NaNO_2$. Indigotine is converted to the pale yellow isatin sulfonate, while amaranth and tartrazine are but little affected. The resultant soln is acidified with HCl, and the dyes are extracted with 2–20 cc portions of amyl alcohol, the isatin compound being less readily extracted by the solvent. To remove amaranth and tartrazine, shake out the amyl alcohol extract with 10 cc portions of 0.25 *N* HCl. Amaranth is much more readily attacked by reducing agents than tartrazine in acid solns than in alkaline. If an alkaline soln of the mixed dyes is carefully treated with a few crystals of $NaHSO_2$, amaranth will be destroyed while the tartrazine will be left practically unaltered. If HCl is added to the mixture, and the dye is extracted with small portions of amyl alcohol, tartrazine can be separated in pure state with 0.25 *N* HCl.

## 10 IDENTIFICATION[10]

The most widely used tests for the identification of coal tar dyes refer to the changes produced with acids and alkalies. Other tests, based upon the behavior with reducing agents, followed perhaps by treatment with oxidants or by separation and identification of the reduction products,[11] and tests based upon oxidation of the dye and treatment of the oxidation products,[12] are generally applicable. Spectroscopic methods are also used.[13]

TABLE I

*Color reactions produced on dyed fibers by various reagents*

| COLORING MATTER | C. I. NO. | S. & J. NO. | STRONG HYDROCHLORIC ACID | CONCENTRATED SULFURIC ACID | 10% SODIUM HYDROXIDE SOLUTION | DILUTE AMMONIUM HYDROXIDE |
|---|---|---|---|---|---|---|
| Rhodamine B | *749* | 504 | Orange | Yellow | Bluer | Bluer |
| Rose Bengal | *779* | 523 | Almost decolorized | Orange | No change | No change |
| Archil | *1242* | 710 | Red | Reddish brown | Violet | Violet |
| Magenta | *677* | 448 | Yellowish brown | Yellowish brown | Decolorized | Paler |
| Acid Magenta | *692* | 462 | Almost decolorized | Yellow | Decolorized | Decolorized |
| Palatine Red | *85* | 62 | Darker | Blue | Dull brown | Little change |
| Bordeaux B | *88* | 65 | Violet | Blue | Brick red | Little change |
| Amaranth | *184* | 107 | Slightly darker | Violet to brownish | Dull brownish to orange red | Little change<br>Little change |
| Azorubine A | *179* | 103 | Little change | Violet | Red | Red |
| Erythrosine | *773* | 517 | Orange-yellow | Orange-yellow | No change | No change |
| Ponceau 6RB | *286* | 169 | Blue | Blue | Dull violet-red | Little change |
| Ponceau 6R | *186* | 108 | Violet-red | Violet | Brown | Orange-red |
| Crystal Ponceau | *89* | 64 | Red | Violet | Dull brown | Little change |
| Ponceau 3R | *80* | 56 | Little change | Little change | Dull orange | Little change |
| Sudan III* | *248* | 143 | Violet, then brown | Green | Violet-red | Little change |
| Safranine | *841* | 584 | Greenish blue | Green | Red | Red |
| Brilliant Scarlet | *185* | 106 | Red | Violet-red | Yellowish brown | Orange-red |
| Ponceau 2R | *79* | 55 | Little change | Little change | Brownish yellow | No change |
| Palatine Scarlet | *77* | 53 | Darker | Violet-red | Brownish yellow | No change |
| Erythrosine G | *772* | 516 | Yellow-orange | Yellow-orange | No change | No change |
| Sudan II* | *73* | 49 | Red | Violet-red | Little change | No change |
| Sudan I* | *24* | 11 | Orange-red | Red | Redder | No change |
| Cochineal | *1239* | 706 | Little change | Little change | Violet-red | Violet-red |
| Bismarck Brown | *331* | 197 | Redder, darker | Browner | Yellower | Yellower |
| Bismarck Brown R | *332* | 201 | Redder, darker | Browner | Yellower | Yellower |
| Orange I | *150* | 85 | Violet | Violet | Red, dark | Red, dark |
| Orange II | *151* | 86 | Red | Red | Dull red | No change |
| Croceine Orange | *26* | 13 | Orange-red | Orange | Slightly darker | No change |
| Orange G | *27* | 14 | Little change | Orange | Dull, brownish red | No change |
| Orthotolueneazobeta-naphthylamine* (Yellow OB) | *61* | . . . | Red | Violet | Little change | No change |

* Oil-soluble.

| | | | | | | |
|---|---|---|---|---|---|---|
| Benzeneazobeta-naphthylamine* (Yellow AB) | *22* | . . . | Red | Violet | Little change | No change |
| Sudan G* | *23* | 10 | Orange-yellow | Brownish yellow | Orange-yellow | No change |
| Butter Yellow* | *19* | 16 | Violet-red | Orange-yellow | No change | No change |
| Aniline Yellow* | *15* | 7 | Violet-red | Orange-yellow | Little change | No change |
| Aminoazoorthotoluene* | *17* | . . . | Dull orange | Orange-yellow | Little change | No change |
| Fluoresceine | *766* | 510 | Little change | Little change | Green fluorescent | Green fluorescent |
| Metanil Yellow | *138* | 95 | Violet-red | Violet | No change | No change |
| Azoflavine | *145* | 92 | Violet-red | Violet-red | Dull brown | Little change |
| Acid Yellow | *16* | 8 | Red | Orange | Little change | No change |
| Brilliant Yellow S | *144* | 89 | Violet-red | Violet-red | Little change | Little change |
| Tartrazine | *640* | 94 | Slightly darker | Slightly darker | Little change | Little change |
| Naphthol Yellow S | *10* | 4 | Almost decolorized | Very pale, dull brown | No change | No change |
| Auramine | *655* | 425 | Decolorized | Almost decolorized | Decolorized | Paler |
| Turmeric | *1238* | 707 | Red | Reddish brown | Orange | Orange |
| Quinoline Yellow | *801* | 667 | Slightly darker | Brownish yellow | Slightly paler | Little change |
| Naphthol Green B | *5* | 398 | Yellowish | Brownish yellow | No change | No change |
| Guinea Green B | *666* | 433 | Pale orange-yellow | Yellowish brown | Decolorized | Decolorized |
| Light Green SF Yellowish | *670* | 435 | Pale orange-yellow | Yellowish brown | Decolorized | Decolorized |
| Fast Green FCF | . . . | . . . | Orange | Green to brown | Blue | Blue |
| Night Green 2B | *667* | 438 | Pale orange-yellow | Yellowish brown | Decolorized | Paler |
| Malachite Green | *657* | 427 | Almost decolorized | Almost decolorized | Decolorized | Decolorized |
| Erioglaucine A | *671* | 436 | Yellow | Pale, dull yellow or brown | Slightly darker | Little change |
| Patent Blue A | *712* | 442 | Pale orange-yellow | Green to brown | Little change | Little change |
| Soluble Blue | *707* | 480 | Paler | Brown | Pale reddish | Almost decolorized |
| Indigotine | *1180* | 692 | Slightly darker | Darker | Greenish yellow | Greenish blue |
| Formyl Violet | *698* | 468 | Pale orange-yellow | Pale, dull orange | Decolorized | Decolorized |
| Methyl Violet | *680* | 451 | Yellowish | Yellowish | Decolorized | Almost decolorized |
| Nigrosine, soluble | *865* | 602 | Dull bluish | Dull greenish | Brownish red, paler | Pale reddish |

### *I. By Color Changes Produced with Acids and Alkalies*

### 11 REAGENTS

(**a**) *Hydrochloric acid.*
(**b**) *Sulfuric acid.*
(**c**) *Sodium hydroxide soln.*—Dissolve 10 g of NaOH in $H_2O$ and dilute to 100 cc.
(**d**) *Ammonium hydroxide.*—Contains 12% $NH_3$ by weight.

### 12 DETERMINATION

Transfer the separated coloring matter to wool (or to silk in the case of oil-soluble dyes) by boiling as directed under **2** (**a**) or **3**. Care should be taken that the final dyeing is made in a soln fairly free from foreign matter such as sugar or aromatic substances, which, adhering to the fiber, may modify the reaction. In most cases the quantity of color available is small and should not be used to dye too large a piece of wool (or silk). Rinse the dyed fiber thoroly in running $H_2O$, dry, cut into small pieces, and place separately in the depressions of a white porcelain spot plate. Moisten the pieces with the reagents described under **11**. (For many coloring matters the hue upon treatment with acids or alkalies varies markedly with the concentration of the reagents and quantity of dye present; therefore the unknown dye should be compared with dyeings of known colors of approximately the same dye concentration as shown by their appearance.)

The table under **13** shows the color changes produced on wool dyed with 0.1–0.5% solns of the respective coloring matters. Included also are the reactions of the oil-soluble colors, but these refer to dyeing on silk. The dyes are arranged approximately according to hue. Brown is classed with orange; black (gray), with violet.

### 14 *II. By Special Tests.*[8]

(**a**) *Oil-soluble dyes* (*yellow AB and yellow OB*).—A method of separating and identifying the 2 permitted oil-soluble dyes is given under **9** (**a**). The alcoholic solns of these dyes become red on treatment with HCl; are unaffected by alkalies; are reduced by $SnCl_2$, $TiCl_3$, and $NaHSO_2$; and the color is not restored to the reduced solns on the addition of $FeCl_3$ or K-persulfate.

(**b**) *Water-soluble dyes* (*amaranth, poncean 3R, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, indigotine, naphthol yellow S, and tartrazine*).—Treatment of these dyes in acid soln with $SnCl_2$, $TiCl_3$, Zn dust, or $NaHSO_2$ decolorizes indigotine, amaranth, tartrazine, ponceau 3R, and orange I. With indigotine the color returns on shaking with air, but more readily on warming or on the addition of $FeCl_3$ or K-persulfate. Excess of the reducing agents must be avoided. With the last 4 named dyes the color is not restored. Dilute solns of light green SF yellowish, guinea green B, naphthol yellow S, and erythrosine become paler or colorless with acid so that the effects of acid-reducing agents are not so readily apparent. Neutral solns of naphthol yellow S are first changed to pink and later decolorized by $NaHSO$. and other reducing agents, the color not returning with air or oxidants. Erythrosine, light green SF yellowish, fast green FCF, and guinea green B become paler with $NaHSO_2$, the color being partially restored upon the addition of K-persulfate.

In hot solns containing an excess of Na-tartrate, the water-soluble dyes are readily decolorized by $TiCl_3$.[14] In the case of indigotine, if the reducing agent has been added carefully and an excess avoided, the blue color readily returns on shaking with air. With erythrosine, light green SF yellowish, fast green FCF, and guinea green B the color is scarcely restored by air, but on cooling and adding K-persulfate it returns imperfectly. The reduction products of the other dyes do not give colored solns again on oxidation, if a slight yellowish or brownish tint that may sometimes appear is disregarded.

(1) *Light green SF yellowish, fast green FCF, and guinea green B* belong to the triphenyl-methane type of dyes. Solns of light green SF yellowish and guinea green B behave similarly with acids, alkalies, and reducing agents, producing a yellow to a greenish yellow with mineral acids, and an almost colorless solution with alkalies as well as with reducing agents. On the other hand, while the reactions of fast green FCF are similar with acids and reducing agents, they differ by producing a deep blue soln with $NH_4OH$ and fixed alkalies, as well as with a 10% soln of Ba-acetate. The easy solubility of these 3 greens in $\alpha$-dichlorhydrin differentiates them from all other permitted dyes. To separate guinea green B from light green SF yellowish and fast green FCF, proceed as follows:

*Light green SF-yellowish and guinea green B.*—Prepare a soln of 250 g of NaCl, 27 g of crystallized Na-acetate, and 24 cc of acetic acid in $H_2O$, and dilute to 1 liter.

To separate and differentiate the 2 green coloring matters add to every 20 cc of dye soln 1 cc of HCl and extract with an equal volume of amyl alcohol. Draw off the lower layer and remove the light green SF yellowish by washing the remaining amyl alcohol portion with equal volumes of the NaCl-sodium acetate soln until no more color is extracted. Dilute the amyl alcohol with an equal volume of gasoline and remove the guinea green B with water.

*Light green SF yellowish and fast green FCF.*—To separate and differentiate proceed as under 9 (3).

(2) *Indigotine* is extracted in small proportions from slightly acid solns by shaking with $\alpha$-dichlorhydrin, from which it may be removed with small portions of 25% salt soln. Most of the other common bluish dyes are triphenyl-methane derivatives and are relatively more soluble in the solvent than in the aqueous layer. Indigo is readily destroyed by boiling with a very small amount of a fixed alkali soln, by which treatment it may be readily eliminated from other coloring matters.

(3) *Ponceau 3R* gives in neutral or faintly acid solns a bluish red, flocculentprecipitate with $BaCl_2$ or Ba-acetate, practically all the dye being removed from soln. Some of the soln obtained in the separation, under 9, may be used in this test, the free HCl first being neutralized with Na-acetate; or better, it may be evaporated to dryness on a steam bath to remove the acid and the residue taken up with a little $H_2O$. A brick red precipitate will be formed on standing, when a neutral soln of the dye is treated with a 20% soln of neutral Pb-acetate. The soln should contain 0.005% or more of the dye.

(4) *Naphthol yellow S*, in solns containing an excess of $NH_4OH$ or $Na_2CO_3$, becomes intensely rose-red on the addition of $NaHSO_3$, the color gradually fading again as complete reduction takes place, also a red coloration is produced if an aqueous soln of the dye is treated with a few drops of 40% $SnCl_2$ and an excess of 20% KOH is added.

(5) *Tartrazine* is characterized by its comparative inactivity towards acids and alkalies, the solution of the dye being hardly altered by the reagents. An alkaline soln of the dye is reduced with $NaHSO_3$ only with difficulty. A concentrated neutral or slightly acid soln of the dye, when reduced with $SnCl_2$ soln or Zn dust and made slightly alkaline and filtered, will develop a purple coloration on standing.

(6) *Orange I* can readily be recognized by its behavior to reagents. With a large excess of HCl it produces a purplish-red; with alkali in large excess it produces a bright red soln.

(7) *Erythrosine* differs from most of the common dyes in that it contains I. To test for I, acidify the soln with $H_2SO_4$, shake with ether, separate the ether soln of the color, and evaporate to dryness in a Pt dish after the addition of a few drops of $Na_2CO_3$ soln or sufficient to form the deep red Na salt. Hold the dish containing the residue in the Bunsen flame until organic matter is destroyed; take up the residue with $H_2O$; acidify with $H_2SO_4$; and test for I in one of the usual ways, such as with Cl water and $CS_2$ or $CCl_4$, or with starch paste and an oxidizing agent. It is useless to test for I with very small quantities of dye, but in most cases sufficient coloring matter can be separated from the food product to give satisfactory results.

## 15 NATURAL COLORING MATTERS

As a class the natural coloring matters show much less tendency to dye animal fiber than do the common synthetic colors. In many cases the crude products used contain a number of colored substances, and a complete separation is not practicable. As dilute solns of most of the natural coloring matters are sensitive to alkalies, and some are sensitive to acids, such reagents must be used with care. Relatively few good tests are known for the common natural colors. Some of their most useful analytical properties[15] are tabulated under **18.**

The properties of pure preparations of the various natural coloring matters are described, for the most part, by Rupe,[16] and by Perkin and Everest,[17] reference being made in these works to the original literature. Properties of the chlorophylls and carotinoids are given by Willstätter and Stoll,[18] those of the coloring matters of the corn flower, rose, pelargona flower, larkspur, cranberry, whortleberry, purple grape, sloe, cherry, plum, radish, and red beet are described by Willstätter and coworkers.[19]

### 16 SEPARATION

(a) *By extraction with ether from neutral solns.*—From neutral solns ether extracts carotin, xanthophyll (the pigments found in leaves, fats and oils, egg yolk, carrots, etc.), the coloring matter of tomatoes and paprika, and green chlorophyll. The coloring matter remains in the ether soln on shaking with normal NaOH soln or normal HCl, no apparent change taking place, altho chemically the substances may be altered more or less by this treatment.

(b) *By extraction with ether from acid solns.*—From slightly acid solns ether extracts very readily and completely the coloring matter of alkanet, annatto, turmeric, and the red dyewoods, sandalwood, camwood, and barwood. It extracts in large proportions the flavone coloring matters of fustic, Persian berries and quercitron (after hydrolysis), as well as the coloring matter of Brazilwood and the green derivatives formed from chlorophyll by alkaline treatment. It extracts in relatively small quantity the coloring matters of logwood, archil, saffron, and cochineal. The coloring matters of this group are readily removed from ether by shaking with alkaline solns, but in most cases they rapidly undergo chemical change.

(c) *By extraction with amyl alcohol from acid solns.*—From slightly acid solns amyl alcohol extracts largely the coloring matters of logwood, archil, saffron, and cochineal. Amyl alcohol extracts in relatively small proportions caramel and the anthocyans constituting the red coloring matter of the most common fruits.

### IDENTIFICATION

#### *I. By color changes produced with various reagents*

#### 17 SOLUTIONS REQUIRED

(a) *Hydrochloric acid.*

(b) *Sodium or potassium hydroxide soln.*—10%.

TABLE II

*Reaction of certain natural coloring matters to common reagents*

| COLORING MATTER | STRONG HYDROCHLORIC ACID | 10 PER CENT SODIUM HYDROXIDE SOLUTION | SODIUM HYPOSULFITE | 0.5 PER CENT FERRIC CHLORIDE SOLUTION | 10 PER CENT ALUM SOLUTION | 5 PER CENT URANIUM ACETATE SOLUTION | CONCENTRATED SULFURIC ACID ON DRY COLOR |
|---|---|---|---|---|---|---|---|
| Logwood | Deep red with excess of acid | Violet to violet-blue | Almost decolorized, color returning imperfectly by reoxidation | Dark shades of violet, brown or black (the first hue often evanescent) | Rose-red (change rather slow) | Violet, quickly fading | Red, changing to yellow |
| Red woods (Brazilwood, Sandalwood, Camwood and Barwood) | Deep red with excess of acid | Violet-red | ........... | Dark shades of violet, brown or black (the first hue often evanescent) | Rose-red (change rather slow) | ........... | ........... |
| Anthocyans of red fruit colors | ........... | Change to green, dull blue or slate color, usually very quickly, becoming browner by oxidation | Anthocyanidins derived by hydrolysis, almost completely decolorized | ........... | ........... | ........... | ........... |
| Alkanet | ........... | Deep blue | ........... | ........... | ........... | Yellowish green | Violet-blue |
| Archil | Little or no change | Blue | Decolorized, color returning when shaken with air. Reaction more easily seen in alkaline solution | ........... | ........... | ........... | Violet-blue |
| Cochineal | Little or no change | Violet | No marked change | Slightly darker | ........... | Green | ........... |
| Annatto | Remains orange. Little change | ........... | Little affected | No marked change. Perhaps somewhat browner | ........... | ........... | Blue |
| Turmeric (solution in ether or alcohol characterized by pure yellow color and light green fluorescence) | Orange-red or carmine-red on addition of several volumes of concentrated acid | Orange-brown | Little affected | No marked change. Perhaps somewhat browner | Little change | Somewhat browner | Red |
| Flavone colors of fustic, Persian berries, quercitron, etc. | Becomes intensely yellow with 2–4 volumes of concentrated acid | Bright yellow | Little affected | Olive-green or black colorations | More strongly yellow; fustic, developing a green fluorescence | Orange colorations | Yellow to orange |
| Saffron | Little or no change | Remains yellow | Little affected | No marked change. Perhaps somewhat browner | Little change | Not affected | Blue |
| Carotin and Xanthophyll | Little change. Perhaps slightly paler | Little or no change | Little affected | ........... | ........... | ........... | Blue, reaction obtained with difficulty |
| Green Chlorophyll | More brownish | "Brown phase reaction," **20** | ........... | ........... | ........... | ........... | ........... |
| Caramel | Little or no change | Little change or slightly deeper brown | Slightly paler | No change | ........... | ........... | ........... |

(c) *Sodium hyposulfite* ($NaHSO_2$).

(d) *Ferric chloride soln.*—0.5%. Freshly prepared. It may also be made by diluting a 10% stock soln.

(e) *Potassium or ammonium alum soln.*—10%.

(f) *Uranium or sodium uranium acetate soln.*—5%.

(g) *Sulfuric acid.*

### 19 DETERMINATION

Evaporate to dryness the ether solns obtained under **16** (a) and **16** (b), warm the residue with a little alcohol, and dilute with $H_2O$. Dilute the amyl alcohol solns obtained under **16** (c) with $H_2O$. To portions of these somewhat purified solns of the coloring matter apply the reagents in the following manner:

*Hydrochloric acid.*—Add the strong acid to the soln, first 1 or 2 drops, then a large excess equal to 3–4 times the volume of the soln.

*Sodium or potassium hydroxide.*—Make the soln slightly alkaline by adding a drop of the 10% NaOH or KOH soln.

*Sodium hyposulfite.*—Add a small crystal of the $NaHSO_2$.

*Ferric chloride.*—Add a small quantity of the 0.5% $FeCl_3$ soln to the soln to be tested very carefully, a small drop at a time, as the colorations are not obtained in some cases when an excess is used.

*Alum.*—Add to the test soln $\frac{1}{5}$ its volume of the 10% K- or $NH_4$-alum soln.

*Uranium acetate.*—Add the 5% U-acetate soln dropwise to the soln to be tested.

*Sulfuric acid on the dry color.*—Evaporate a small quantity of the soln or of the coloring matter in a porcelain dish. Cool thoroly and treat the dry residue with 1 or 2 drops of cold $H_2SO_4$. The colorations are in some cases extremely fugitive, and they may be observed only the instant the acid wets the residue.

Table **II**, under **18**, shows the behavior of certain of the natural coloring matters when treated in the manner described above.

### 20 *II. By Special Tests*

*Chlorophyll.*—The "brown phase reaction"[20] may be useful for the characterization of chlorophyll, when this has not been previously treated with alkalies. Treat the green ether or petroleum ether soln of the coloring matter with a small quantity of 10% soln of KOH in methyl alcohol. The color becomes brown, quickly returning to green.

**21**

*Annatto*[21].—Pour on a moistened filter an alkaline soln of the color obtained by shaking out the oil or melted and filtered fat with warm, dilute NaOH soln. If annatto is present, the filter paper will absorb the color, so that when washed with a gentle stream of $H_2O$ it will remain dyed a straw color. Dry the filter and add a drop of $SnCl_2$ soln. If the color turns pink, the presence of annatto is confirmed.

**22**

*Turmeric.*—Treat an aqueous or dilute alcoholic soln of the color with HCl until the shade just begins to appear slightly orange. Divide the mixture into two parts and add some $H_3BO_3$ powder or crystals to one portion. A marked reddening will be quickly apparent, best seen by comparison with the portion to which the $H_3BO_3$ has not been added. The test may also be made by dipping a piece of filter paper in the alcoholic soln of the coloring matter, drying at 100°, then moistening with a weak soln of $H_3BO_3$ to which a few drops of HCl have been added. On drying again a cherry-red color will be developed.

23

*Cochineal.*—When the presence of cochineal is suspected, acidify the mixture with $\frac{1}{3}$ its volume of HCl and shake with amyl alcohol. Wash the amyl alcohol soln of the coloring matter 2–4 times with equal volumes of $H_2O$ to remove HCl, etc. Dilute the amyl alcohol with 1–2 volumes of gasoline and shake with a few small portions of $H_2O$ to remove the color. Divide the combined aqueous extracts into 2 portions. To the first add, dropwise, 5% U-acetate soln, shaking thoroly after each addition. In the presence of cochineal a characteristic emerald-green color is produced.[22] The green coloration with U-salts is not developed in the presence of much free acid. Therefore, add a little Na-acetate before making this test, or a correspondingly large quantity of U-acetate must be added. To the second portion add 1 or 2 drops of $NH_4OH$; in the presence of cochineal, a violet coloration results. This, however, is not so characteristic as the first test as many fruit colors give almost identical reactions. Cochineal is not decolorized by $NaHSO_2$ either in an acid, neutral or alkaline soln (differs from orchil).

As cochineal lakes often contain tin, further examination for this metal should always be made when water-insoluble cochineal compounds seem to be present.

24

*Orchil.*—This coloring matter is either sulfonated or unsulfonated. Unsulfonated orchil is readily extracted by amyl alcohol from a weak acid soln, while the extraction of the sulfonated color is incomplete even from a strongly acidified soln. The behavior of the color towards acids and alkalies is similar to cochineal, e. g., HCl produces a yellow shade and alkalies produce a bluish shade. $NaHSO_2$ reduces orchil, but the color is restored by air oxidation (differing from cochineal). The characteristic property of orchil is to dye, strip, and redye wool readily.

25

*Caramel.*—A number of tests have been developed for this coloring matter, most of them being based upon the insolubility in ether, $CHCl_3$, or amyl alcohol. Probably the most sensitive test is the Woodman-Newhall[23] modification of Amthor's test with a slight deviation. To 10–20 cc of a neutral soln of the color in a small centrifuge tube add 2 cc of 5% $ZnCl_2$ and 2 cc of 2% KOH soln, stir well, and centrifugalize. Pour off the liquid, and to the magma add 25 cc of boiling $H_2O$. Mix, centrifugalize, and pour off liquid. Repeat this operation until the aqueous wash liquor is colorless. Dissolve the precipitate with 15 cc of 10% acetic acid, concentrate, neutralize carefully, and filter. Divide into 2 portions. To one add 3–5 volumes of paraldehyde in a 50 cc glass-stoppered cylinder, and just sufficient absolute alcohol to form a homogeneous soln (avoid excess). Caramel will be indicated by the formation of a brownish precipitate on standing. To the other portion of the caramel soln add an equal volume of a freshly prepared reagent consisting of phenylhydrazin hydrochloride—2 parts, Na-acetate—3 parts, water—20 parts. A dark brown precipitate is formed in the presence of caramel.

## COMMERCIAL COAL TAR FOOD COLORS[24]

26

### PREPARATION OF SAMPLE

Thoroly mix and without interruption weigh out the portions required for the determinations to be made. If the weighing cannot be made directly into the dish in which the determination is to be made, use weighing bottles for this purpose, placing in each a quantity approximating the weight called for, and weigh immediately.

## 27 MOISTURE

(a) *For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and indigotine.*—Weigh approximately 2 g of the sample in a weighed Al dish 2 inches in diameter or in a weighing bottle of the same diameter, dry in an air oven at 135° for 6 hours or overnight, cool over $H_2SO_4$ in a desiccator, and weigh. Heat again for 1 hour, cool in the desiccator, and weigh. Repeat the heating and weighing at hour intervals until the weight becomes constant. Report the loss in weight as moisture.

(b) *For yellow AB and yellow OB.*—Proceed as directed under (a), heating the dye to 80° instead of to 135°.

## WATER-INSOLUBLE MATTER

### 28 APPARATUS

*Prepared Gooch crucible.*—Digest a good grade of retentive asbestos with HCl (1+3), wash free from acid, and elutriate to remove fine particles; pour a sufficient quantity into a Gooch crucible placed in a filter flask to make a mat $\frac{1}{8}$ inch thick when packed. Using gentle suction, pack the asbestos down evenly with a tamping rod and then remove the Gooch from the filter flask. Loosen the mat around the edges with a thin narrow blade or pin, take out of Gooch, replace in an inverted position, pack down tightly, and add more of the asbestos suspension until a well packed mat 1 inch thick is obtained. Wash with hot $H_2O$, dry, ignite, rewash, dry at 100–105°, cool in a desiccator containing $CaCl_2$, and weigh. Repeat the washing, heating, and drying until constant weight is obtained.

### 29 DETERMINATION

(a) *For amaranth, erythrosine, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, and fast green FCF.*—Dissolve 5 g of dye in 200 cc of hot $H_2O$ and allow the soln to cool to room temp. Filter thru the prepared Gooch crucible, wash with cold $H_2O$ until all dissolved dye has been removed, dry at 135°, cool in a desiccator containing $CaCl_2$, and weigh. Report the increase in weight as total insoluble matter.

(b) *For ponceau 3R, orange I, and indigotine.*—Dissolve 5 g of dye in hot $H_2O$, using 250 cc for ponceau 3R and orange I, and 500 cc for indigotine, and boil, with frequent stirring, for 3 min. Cool the soln to room temp. and filter with moderate suction. Wash with cold $H_2O$, dry, cool, and weigh as directed under (a).

## 30 NON-VOLATILE WATER-INSOLUBLE MATTER

*For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF and indigotine.*—Incinerate the Gooch containing the total insoluble matter, **29**, at a low red heat until all the organic matter has been volatilized. Cool in a desiccator containing $CaCl_2$ and weigh.

## SODIUM CHLORIDE

### REAGENTS

All reagents must be halogen free.

*Sulfur dioxide soln.*—Saturate ice-cold distilled $H_2O$ with sulfur dioxide. Keep the soln stoppered and in a cold place.

### 31 DETERMINATION

(a) *Applicable to ponceau 3R, amaranth, orange I, tartrazine, guinea green B, light* *green SF yellowish, fast green FCF, and indigotine.*—Thoroly mix 5 g of dye with 4–6 g of $K_2CO_3$ or $Na_2CO_3$ in a 50 cc Pt or Ni crucible and moisten with $H_2O$ or 50% alcohol. Cover evenly with about 1 g of the powdered carbonate, dry, and ignite at low red heat until organic matter is destroyed. Allow to cool and add enough $H_2O$ to form a thin paste. If any lumps are present, break them up with a glass rod in order to produce a uniform suspension. Wash the mixture into a 250 cc volumetric flask with 100–150 cc of hot $H_2O$ and allow to stand until all soluble salts are dissolved and the mixture is cold. Dilute to the mark with $H_2O$, mix thoroly, and filter thru a dry paper.

Place a 200 cc portion of the filtrate in a 600 cc beaker and add enough of a 6-7% soln of $KMnO_4$ to oxidize the sulfides and produce a permanent pink color. Add about 50 cc of $H_2O$ and a slight excess of 10% $AgNO_3$ soln (usually 6–8 cc is sufficient). Partially cover the beaker with a watch-glass and acidify the soln by carefully adding about 12 cc of $HNO_3$. Heat nearly to boiling, then add the saturated $SO_2$ soln. Boil until any excess of $SO_2$ is removed, cool, filter thru a weighed Gooch crucible, wash the precipitate of AgCl with hot $H_2O$, dry crucible and its contents at 140–150°, cool in a desiccator, and weigh. Calculate to percentage of NaCl.

(b) *For erythrosine.*—In a 500 cc volumetric flask dissolve 5 g of the dye in 400 cc of $H_2O$. Precipitate the color acid by adding a mixture of 2 cc of $HNO_3$ and 10–20 cc of $H_2O$, dilute to 500 cc, mix, and filter thru a dry paper. Treat 200 cc of the filtrate with slightly more 10% $AgNO_3$ soln than is required to precipitate the halogens present, add 5 cc of $HNO_3$, and heat to boiling. Cool, collect the precipitate in a weighed Gooch crucible, wash, dry, and weigh as directed under (a). If NaI is present, determine as directed under **62**, and subtract the weight of AgI from the weight of the precipitate. Calculate the percentage of NaCl from the net AgCl.

(c) *For naphthol yellow S.*—Dissolve 5 g of the dye in 250 cc of $H_2O$ and filter if necessary. Add 5 cc of $HNO_3$ and precipitate the chloride by adding a slight excess of 10% $AgNO_3$ soln. Boil for a few min., cool, and filter thru a weighed Gooch crucible. Wash, dry, and weigh the precipitate and calculate as directed under (a).

(d) *For ponceau 3R.*—Dissolve 5 g of the dye in 150 cc of hot $H_2O$, wash into a 250 cc volumetric flask, and add 25 cc of a 10% soln of $Ba(NO_3)_2$. Cool the mixture, make up to the mark, mix, and filter thru a dry paper. Acidify 100 cc of the filtrate (representing 2 g of dye) with $HNO_3$ and treat with a slight excess of 10% $AgNO_3$ soln. Collect the filtrate on a weighed Gooch crucible, wash, cool in a desiccator, weigh, and calculate as directed under (a).

(e) *For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and indigotine.*—In a Ni crucible mix 1 g of the dye with about 15 g of pure $Na_2O_2$. Place the cover on the crucible and start the fusion by introducing a red-hot iron wire thru a hole previously bored in the cover. When the reaction is complete, allow the crucible to cool. (If the fusion is too rapid, repeat the entire procedure, using a larger proportion of $Na_2O_2$.) Wash the cover into a 600 cc beaker, using 50–75 cc of $H_2O$. Place the crucible on its side in the beaker and add $H_2O$ until the crucible is about half covered. When the mixture is dissolved, remove and wash the crucible over the beaker. Neutralize the soln with $HNO_3$, adding 1–3 cc in excess. Cool, transfer to a 6-inch porcelain evaporating dish, and add 19 cc of 0.1 $N$ $AgNO_3$ soln and 5–10 cc of 10% ferric alum soln. Titrate the soln with 0.1 $N$ $NH_4$- or K-thiocyanate, adding 1 or 2 drops after the red end point has been reached. Add another cc of $AgNO_3$, making 20 cc in all. Filter the soln thru a Büchner funnel and wash the precipitate with a little $H_2O$ slightly acidified with $HNO_3$. Return the filtrate and washing to the evaporating dish and titrate to the end point with $NH_4$- or K-thiocyanate. Calculate to percentage of NaCl.

## 32 SODIUM SULFATE

(a) *For ponceau 3R, amaranth, orange I, tartrazine, and indigotine.*—Transfer to a 250 cc volumetric flask that volume of a water soln of the sample which contains 5 g of the dye; add $H_2O$, if necessary, to bring the volume to 200 cc; and heat on the steam bath. Add pulverized NaCl as follows: For amaranth and tartrazine, 70 g; for ponceau 3R, orange I, indigotine, 50 g. Stopper the flask and shake at frequent intervals for an hour. (To hasten the precipitation the soln may be cooled in ice $H_2O$.) Dilute to the mark with saturated NaCl soln, shake, and filter on a dry 18 cm paper. To 100 cc of the filtrate add 200 cc of $H_2O$ and 1 cc of HCl (1+9), heat to boiling, and add a slight excess of hot 10% $BaCl_2$ soln. Allow to stand overnight, filter thru a weighed Gooch crucible, wash the precipitate of $BaSO_4$ thoroly with hot $H_2O$, dry, ignite, cool in a desiccator containing $CaCl_2$, and weigh. Calculate the weight of $Na_2SO_4$ equivalent to the $BaSO_4$ obtained.

(b) *For erythrosine.*—To such a volume of a water soln of the sample as contains 1 g of dye add $H_2O$, if necessary, to bring the volume to 100 cc and then add 50 cc of $HNO_3$ (1+49). Shake, and let settle. Filter off the dye and wash once, collecting the washings with the filtrate. Redissolve the dye by passing thru the filter paper as little $NH_4OH$ (1+1) as will effect soln. Neutralize the base and reprecipitate the color acid by means of the $HNO_3$ (1+49). Filter off the dye and wash once, collecting the filtrate and washings with those obtained after the first precipitation of color acid. [A 100 cc aliquot free of the color acid **31** (b) may be substituted.] Precipitate and determine the $BaSO_4$ as directed under (a).

(c) *For light green SF yellowish and fast green FCF.*—Transfer to a 250 cc flask that volume of a soln which contains 5 g of the dye; add $H_2O$, if necessary, to bring to a volume of about 200 cc, and heat on the steam bath. Add 5 g of phosphotungstic acid and shake at intervals until dissolved. Then add 50 g of pure pulverized NaCl, shaking at intervals to dissolve the salt. Cool, dilute to mark with saturated pure NaCl soln, shake, and filter. To 100 cc of the filtrate add 200 cc of $H_2O$ and 1 cc of HCl (1+9) and determine $BaSO_4$ as directed under (a).

(d) *For naphthol yellow S.*—To a volume of a water soln of the dye that contains 5 g of the sample in a 500 cc volumetric flask, add $H_2O$, if necessary, to bring to a volume of about 300 cc. Add hot saturated KCl soln to bring to the 500 cc mark. Shake frequently until practically all the dye is precipitated. After the mixture is cold dilute with $H_2O$ to 500 cc. Shake, and filter thru a dry paper. Complete the determination as directed under (a) beginning with "To 100 cc of the filtrate add 200 cc of $H_2O$ and 1 cc of HCl (1+9)."

(e) *For guinea green B.*—Transfer to a 500 cc volumetric flask that volume of a water soln of the sample which contains 5 g of the dye, and add $H_2O$, if necessary, to bring the volume to 400 cc. Add 8 cc of $NH_4OH$ and 125 g of NaCl (free from sulfates), and dilute to 500 cc with a saturated soln of NaCl. Shake vigorously to precipitate the dye and filter thru a dry paper. Neutralize 200 cc of the filtrate with HCl (1+9) and add 5 cc in excess. Complete the determination as directed under (a), beginning with "heat to boiling, and add a slight excess of hot 10% $BaCl_2$ soln."

## 33 SULFATED ASH

(a) *For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S tartrazine, guinea green B, light green SF yellowish, fast green FCF, and indigotine.*—Weigh accurately in a weighing bottle about 5 g of the dye and transfer it to a Pyrex Kjeldahl flask, washing out the weighing bottle with a little $H_2O$. Destroy the organic matter to a convenient extent by digestion, using 15 cc of $H_2SO_4$ and adding $HNO_3$ as required. As the bulk of the $HNO_3$ is driven off, lower the flame to avoid reaction on the glass. Transfer the mixture to a weighed Pt dish and heat over a ring burner, using at first a low flame at a safe distance below the dish, increasing the flame, and bringing it closer to the dish by gradual steps. Thus continue the destruction of the organic matter and the volatilization of the acids. Continue the heating until the production of acid fumes decreases. If C remains, remove the flame; let the mass cool a little; and add $H_2SO_4$, dropwise, until the mass is moistened. Repeat the treatment until the C is burned off and the ash is white or reddish. Heat carefully with a blast lamp until fusion takes place with the production of a clear liquid free from bubbles. Cool in a desiccator and weigh. After deducting the weight of $Na_2SO_4$ equivalent to the inorganic Na salts (chlorides, sulfates, carbonates, etc.) found in the other determinations, calculate to percentage of metallic Na combined in the dye.

**(b)** *For yellow AB and yellow OB.*—In a weighed Pt dish weigh 5 g of the dye, heat at a low temp. until most of the dye has been volatilized, moisten the residue with $H_2SO_4$, and complete the determination as directed under **(a)**, beginning with "Repeat the treatment until the C is burned off."

### **34** HEAVY METALS

**(a)** *For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and indigotine.*—Moisten the sulfated ash obtained under **33** with a few cc of HCl and evaporate to dryness on the steam bath. Warm the residue with 20 cc of HCl (1+19) until all soluble material has dissolved, transfer to a 100 cc volumetric flask, dilute to 100 cc, mix, and filter thru a dry paper. Reserve two 40 cc aliquots for the determination of Al, Ca, Fe, and Mg. Pour 20 cc of the filtrate into a test tube and pass in a washed stream of $H_2S$ for 30 min. No turbidity other than that due to precipitated S should appear. If a colored precipitate is formed, filter and test it for Pb, Cu, and Sn. Make the filtrate slightly alkaline with $NH_4OH$ (1+1). A white precipitate indicates Zn; a marked coloration indicates that the quantity of Fe should be determined.

**(b)** *For yellow AB and yellow OB.*—The color of the sulfated ash obtained under **33** shows whether it is mainly $Fe_2O_3$ or $SiO_2$. In either case fuse it with 1 g of $K_2CO_3$ until the silicates have been decomposed. Moisten the residue with 2 or 3 cc of HCl, evaporate to dryness on the steam bath, and treat as directed under **(a)**. If only a trace of $Fe_2O_3$ or $SiO_2$ is present in the original ash, the fusion with $K_2CO_3$ may be omitted.

## LEAD[25]

(Applicable to all permitted dyes)

### **35** REAGENTS

**(a)** *Ammonium acetate.*—Dissolve 200 g of pure $NH_4$-acetate in $H_2O$ and dilute to 500 cc.

**(b)** *Ammonium acetate soln.*—Dilute 50 cc of the strong $NH_4$-acetate to 500 cc.

### **36** DETERMINATION

Place 5 g of the dye in a tall form, 500 cc Pyrex beaker, cover with a watch-glass, add 15 cc of $HNO_3$, and let boil, or heat gently till the rapid evolution of brown fumes has ceased. Add 15 cc of $H_2SO_4$ and continue the heating. Add small quantities (1–2 cc) of $HNO_3$ at intervals until the organic matter is destroyed and the soln is colorless or at most a pale yellow. Continue the heating, with the evolution of dense white fumes, until a very small quantity (3–5 cc) of soln remains in the beaker. Cool the soln, which should form white or pale yellow crystals, and add 15–20 cc of $H_2O$. Re-evaporate the soln thus formed down to white fumes, cool, take up in 100 cc of $H_2O$, add 100 cc of 95% ethyl alcohol, and let stand overnight. Filter out the precipitate of $PbSO_4$, which may be present in such a small quantity as to escape detection with the naked eye, and wash thoroly with 50% ethyl alcohol (about 100 cc is the usual amount of 50% alcohol used). (Two 9 cm C. S. & S. No. 590 filter papers, or a suitable, fritted glass crucible, have been found satisfactory for retaining the $PbSO_4$.)

Place the filter paper in a small beaker, add 20 cc of strong $NH_4$-acetate, and heat to boiling, breaking up the paper with a glass rod. Filter thru a C. S. & S. No. 590 9 cm paper, or thru a fritted glass crucible, into a 100 cc colorimeter tube and wash with dilute $NH_4$-acetate soln until the 50 cc mark is reached. If desired, filter into a 50 cc volumetric flask and take an aliquot portion to be used in the colorimeter tube. Prepare standards containing known quantities of Pb for comparison. To these add the same quantity of $NH_4$-acetate as was used with the sample and dilute all tubes to a definite volume with $H_2O$. To each tube add 2 or 3 drops of glacial acetic acid and 10 cc of freshly prepared $H_2S$ water. Shake the tubes to insure thoro mixing and estimate the quantity of Pb by comparison with the standards. Blanks should not be over 4 p.p.m. when using the best reagents obtainable.

### 37 IRON, ALUMINUM, CALCIUM, AND MAGNESIUM[26]

*For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, yellow AB, yellow OB, guinea green B, light green SF yellowish, fast green FCF and indigotine.*—To one of the two portions reserved under **34**, add 5 g of $NH_4Cl$ and neutralize with $NH_4OH$, boiling to drive off any excess. If the precipitate is very slight, it may be disregarded; otherwise, filter thru a quantitative paper, wash (reserving the filtrates and washings), and ignite paper and precipitate in a weighed crucible. Weigh the mixture of $Fe_2O_3$ and $Al_2O_3$. Place the mixed oxides in a 500 cc Erlenmeyer flask and dissolve in a $HNO_3$ and HCl mixture, boiling to drive off Cl. Add $H_2O$ to bring the volume to about 75 cc and add $NH_4OH$ to incipient precipitation. Dissolve the precipitate with as little HCl as possible, cool, and titrate the ferric iron present with 0.1 *N* $TiCl_3$ soln, **46**, using 5 g of $NH_4CNS$ as indicator. Calculate the Fe as $Fe_2O_3$. To calculate the quantity of $Al_2O_3$, deduct the weight of ferric oxide from the total weight of mixed oxides. From the weights of the oxides calculate the percentage of metallic iron and Al. Pass a washed stream of $H_2S$ into the alkaline filtrate from the Fe- and Al- hydroxides. A white precipitate indicates the presence of Zn.

To the other reserved portion add 250 cc of $H_2O$ to insure a low concentration of Mg, if present. Heat to boiling and add 3.5 g of $NH_4Cl$ and enough $NH_4OH$ soln (1+99) to make the soln barely alkaline. Filter off the precipitated hydroxides of Fe and Al. Wash and discard the precipitate. Heat the combined filtrate and washings to boiling and add 1 g of $NH_4$-oxalate. After cooling and letting stand for an hour, filter thru an asbestos mat prepared on a small Witt plate in a glass funnel and wash with very little $H_2O$, reserving the combined filtrate and washings. Place the mat in a beaker, add 100 cc of $H_2O$ and 2 cc of $H_2SO_4$, heat gently until the Ca-oxalate dissolves, and titrate with 0.1 *N* $KMnO_4$ soln. Calculate as metallic Ca.

Heat to boiling the reserved filtrate and washings and add a *N* soln of $NaNH_4HPO_4$ until there is no further precipitation. While stirring add about $\frac{1}{3}$ the volume of $NH_4OH$ (1+9). Let stand 3 hours, filter thru an ashless paper, and wash with $NH_4OH$ (1+49). Ignite the filter and precipitate in a weighed crucible, cool in a desiccator, and weigh the $Mg_2P_2O_7$. Calculate as metallic Mg.

## ARSENIC

### *I. By Direct Precipitation*

38 REAGENTS

(a) *Ammonium hydroxide.*—Arsenic free and containing not less than 26% by weight of $NH_3$.

(b) *Hydrochloric acid* (1+3).—Should be As-free.

(c) *Sodium phosphate soln.*—As-free and containing 100 g of the crystallized salt per liter or an equivalent quantity of phosphoric acid.

(d) *Magnesia mixture.*—As-free and containing 55 g of hydrated $MgCl_2$, 55 g of $NH_4Cl$, and 88 cc of $NH_4OH$ per liter.

The other reagents and solns used are described under **XXIX, 1.**

39 APPARATUS

The apparatus used is described under **XXIX, 2.**

40 DETERMINATION

(a) *For amaranth, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, and fast green FCF.*—Dissolve 10 g of the dye in 250 cc of $H_2O$ and add 10 cc of strong Br water. Make the mixture alkaline with 1–2 cc of the strong $NH_4OH$; then add 20 cc of the Na-phosphate soln and a slight excess of the Mg mixture. (The quantity of Mg mixture used must be from 1–5 cc in excess of that required to precipitate the phosphate completely, as ascertained previously by experiment.) Add the Mg mixture to the dye mixture slowly, stirring the soln during the addition. Add 10 cc of the $NH_4OH$ and allow the mixture to stand for at least 30 min. Filter thru an 18 cm paper and wash with $NH_4OH$ (1+9) until practically all dye is removed; then wash with about 5 cc of $H_2O$. Allow the filter containing the washed precipitate to drain for 15–30 min. to remove most of the adhering liquid. Finally dissolve the Mg-$NH_4$-phosphate and arsenate by pouring 40 cc of the HCl (1+4) over the filter in small portions and letting it drain into the generator bottle. Complete the determination as directed under **XXIX, 4,** beginning with, "add 5 cc of the KI reagent."

(b) *For erythrosine.*—Dissolve 18 g of the dye in 425 cc of $H_2O$ and add 5 cc of of Br water and 20 cc of the HCl (1+3). Mix, filter, and treat 250 cc of the filtrate (corresponding to 10 g of dye) with the strong $NH_4OH$ soln, using a quantity sufficient to render it slightly alkaline (usually about 5 cc). Complete the determination as directed under (a), beginning with "then add 20 cc of the Na-phosphate soln."

### 41 *II. After Treatment with Nitric Acid*

(a) *For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, and indigotine.*—Place 12.5 g of the powdered dye in a 600 cc Pyrex beaker; add 25 cc of the strong $HNO_3$; and, if the dye tends to form a clot or cake, stir the mixture thoroly. Boil for about 5 min., add 150–200 cc of $H_2O$, and dilute the mixture to 250 cc in a volumetric flask. Pour the mixture back into the beaker, allow to stand for a few min., and then filter thru an 18 cm paper. Treat 200 cc of the filtrate (corresponding to 10 g of dye) with the strong $NH_4OH$, using a measured quantity sufficient to make the soln slightly alkaline (approximately 25 cc). Complete the determination as directed under **40** (a), beginning with "then add 20 cc of the Na-phosphate soln."

(b) *For yellow AB and yellow OB.*—In a 400 cc beaker mix thoroly 12.5 g of the powdered dye with 200 cc of $HNO_3$ (1+9), heat to boiling for 5–10 min., allow to cool, add 25 cc of the strong $NH_4OH$, dilute to 250 cc in a volumetric flask, and filter. Determine the As in a 200 cc portion of the filtrate (corresponding to 10 g of dye) as directed under **40** (a), beginning with "then add 20 cc of the Na-phosphate soln."

### 42 *III. As Total Arsenic*

*For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, yellow AB, yellow OB, guinea green B, light green SF yellowish, fast green FCF, and indigotine.*—Weigh 10 g of the dye into a 600 cc Kjeldahl flask or tall 600 cc beaker provided with a cover. Treat with 15 cc of the concentrated $H_2SO_4$ and 25 cc of the $HNO_3$ and digest slowly under a hood until the $HNO_3$ has been decomposed or volatilized and the mixture turns dark. Cautiously add a few cc of $HNO_3$ to the hot mixture, which will again become light yellow or orange, and heat to charring. Repeat the $HNO_3$ treatment until the soln no longer shows a tendency to darken and remains yellow or colorless when evaporated until $SO_3$ fumes are evolved. Allow the completely digested mixture to cool, add 200 cc of $H_2O$, and make slightly alkaline with $NH_4OH$. Determine the As in the soln as directed under **40** (a), beginning with "then add 20 cc of the Na-phosphate soln." The comparatively large quantities of reagents necessary for the destruction of the organic matter will usually contain appreciable quantities of As, for which correction must be made by determinations on blanks.

## ETHER EXTRACTIVES

### 43 REAGENTS

*Washed ether.*—Wash 1 liter of ether with 3 successive 150 cc portions of $H_2O$ immediately before using.

### 44 DETERMINATION

(a) *For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and indigotine.*—Place in a separatory funnel that volume of a soln that contains 10 g of the dye and add water, if necessary, to bring the volume to 200 cc, and, in the case of indigotine, to 500 cc. Extract with 2 successive 100 cc portions of the washed ether, shaking for 1 min. during each extraction. Remove the ether by decantation into a clean funnel and rinse the first funnel with 5 cc of ether, decanting into the second funnel. Reserve the color soln. Wash the combined extracts with 20 cc portions of $H_2O$ until the washings are colorless. Decant the ether into a beaker, rinse the funnel with 5 cc of ether, and decant into the same beaker. Place the beaker in a dust-free atmosphere, allow the ether to evaporate to a volume of 50 cc, and transfer to a weighed flat-bottomed 100 cc dish, previously dried to constant weight over $H_2SO_4$ in a desiccator. Rinse the beaker with 5 cc of ether and drain into the same dish. Let the remainder of the ether evaporate and dry over $H_2SO_4$ to constant weight. The result represents the neutral extract.

To the reserved color soln, add 2 cc of a 10% NaOH soln and extract and rinse with ether. Reserve the color soln. Wash the combined ether extracts and rinsings with 20 cc portions of the dilute NaOH soln until the washings are colorless. Evaporate the ether, dry, and weigh. The result represents the alkaline extract.

To the color soln reserved from the alkaline extraction, add twice the volume of HCl (1+3) necessary to neutralize. Repeat the previous procedure, but do not reserve the color soln. Wash the ether extract with the dilute HCl until the washings are colorless. The result represents the acid extract.

(b) *For erythrosine.*—Determine as directed under (a), omitting the acid extraction.

### 45 SULFUR

*For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and indigotine.*—(1) Determine the S content in 0.2–0.3 g of the dye by the Carius method,[27] using 3 cc of fuming $HNO_3$ and heating the sealed tube to 300° for at least 8 hours; or (2) Place about 0.2 g of the sample in a Parr calorimetric bomb and mix thoroly with approximately 10 g of $Na_2O_2$. Add a few mg of S-free sugar if necessary to aid in igniting the mass. Close the bomb and ignite. When cool, open the bomb, place it in a 400 cc beaker, and cover the beaker with a watch-glass. Dissolve the residue by adding warm $H_2O$ thru the lip of the beaker until the bomb is covered. Acidify the soln cautiously with dilute HCl and filter, if necessary. Determine the $H_2SO_4$ as directed under **32** (a), beginning with "heat to boiling, and add a slight excess of hot 10% $BaCl_2$ soln." Deduct the S equivalent to the $Na_2SO_4$ determined under **32**.

### COLOR ACID AND DYE

#### *I. By Titration with Titanium Trichloride*

### 46 REAGENT

*Standard titanium trichloride soln.*—To 200 cc of the commercial 15% soln of $TiCl_3$, add 150 cc of HCl and dilute to 2 liters. Titrate against 10 cc of 0.1 *N* ferric ammonium sulfate, using 5 g of $NH_4CNS$ as an indicator. Make the soln approximately 0.1 *N*, place in a container with a H atmosphere provision,[28] and allow to stand for 2 days for absorption of residual O. Standardize against 20 cc of 0.1 *N* ferric ammonium sulfate, using 5 g of $NH_4CNS$ as indicator and titrating in a 500 cc Erlenmeyer flask in a protecting stream of $CO_2$. Preserve in an atmosphere of H.[28]

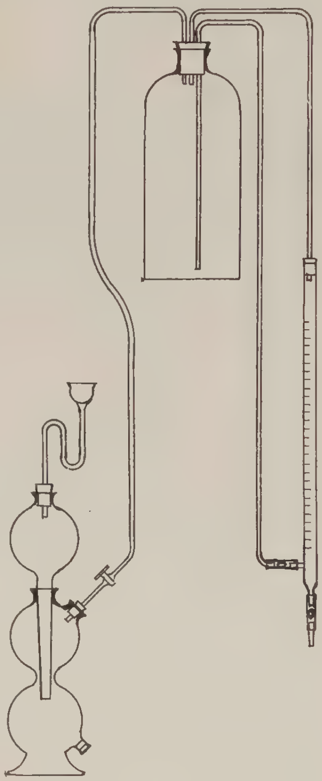

FIG. 20.—APPARATUS FOR TITRATION WITH TITANIUM TRICHLORIDE

### STANDARDIZATION OF SOLUTION

*Method I.*—Prepare a liter of 0.1 *N* $Fe_2(SO_4)_3$ by dissolving ingot iron, Bureau of Standards Sample 55, in dilute $H_2SO_4$, using 30 cc of the concentrated acid. Dilute to about 400 cc, adding slowly, with stirring, a soln of pure $KMnO_4$ (3.16 g dissolved in about 200 cc $H_2O$) until a faint but perceptible reddish tint results. The last few cc should be added dropwise. Cool and dilute to 1 liter. Measure 20 cc of the 0.1 *N* $Fe_2(SO_4)_3$ into a 500 cc flask, pass in a strong stream of $CO_2$, and add the $TiCl_3$ rapidly until near the end point. Add 5 g or pure $NH_4CNS$ and resume the addition of $TICl_3$ carefully until the red color just disappears.

*Method II*—Make up a 0.1 *N* soln of $KMnO_4$ and standardize carefully, using Na-oxalate, Bureau of Standards Sample 40, according to the directions supplied with the sample. Weigh 3 g of ferrous ammonium sulfate and transfer to a 500 cc flask. Introduce a stream of $CO_2$ and add 50 cc of recently boiled $H_2O$ and 25 cc of 40% (by weight) $H_2SO_4$. Then, without interrupting the current of $CO_2$, add rapidly 40 cc of the standardized $KMnO_4$. Add $TiCl_3$ until near the calculated end point. Then add quickly 5 g of $NH_4CNS$, and complete the titration. Run a blank on 3 g of ferrous ammonium sulfate, using the same quantities of $H_2O$, acid, $NH_4CNS$, and the current of $CO_2$.

### INDICATOR

For many dyes the $TiCl_3$ titration end point is indicated by a sharp decolorization. For some dyes the change is so gradual that an excess of $TiCl_3$ (not more than 0.3 cc of approximately 0.1 *N* soln) is required, and a suitable standard soln of some other dye must be used for the back titration, methylene blue serving well for this purpose. In other cases it is better to use an indicator which is reduced after the original dye has reacted with the $TiCl_3$. Thus a known quantity of light green SF yellowish serves well for this purpose.

**47**

Prepare a dye solution of such strength that 50 cc will require approximately 20 cc of the standard $TiCl_3$ soln for its reduction. See table below.

*Quantities of color acids and of pure coal-tar dye equivalent to 1 cc of 0.1 N titanium trichloride solution*

| DYE | MOLECULAR WEIGHT OF COLOR ACID | COLOR ACID EQUIVALENT TO 1 CC 0.1 *N* $TiCl_3$ | DYE EQUIVALENT TO 1 CC 0.1 *N* $TiCl_3$ |
|---|---|---|---|
| Ponceau 3R | 450.4 | 0.01126 | 0.01236 |
| Amaranth | 538.4 | .01346 | .01511 |
| Orange I | 328.3 | .008207 | .008756 |
| Naphthol yellow S | 314.2 | .002618 | .002985 |
| Tartrazine | 468.3 | .01171 | .01336 |
| Guinea green B | 668.5 | .03342 | .03453 |
| Light green SF yellowish | 748.7 | .03743 | .03963 |
| Fast green FCF | 764.5 | .03822 | .040423 |
| Indigotine | 422.3 | .02112 | .02332 |

**48** DETERMINATION

(**a**) *For amaranth and ponceau 3R.*—Place in a 500 cc Erlenmeyer flask a volume of soln that corresponds to 20 cc of 0.1 *N* $TiCl_3$. Add 10 g of Na-citrate and $H_2O$ if necessary to bring the volume to 100 cc. Introduce a stream of $CO_2$ and titrate with the standardized $TiCl_3$, keeping the $CO_2$ flow continuous to the end.

(**b**) *For orange I, tartrazine and indigotine.*—Proceed as directed under (**a**), substituting 15 g of Na-acid tartrate for Na-citrate and bringing the volume to 100 cc. First completely dissolve the tartrate by boiling; then add the dye soln, and boil again under $CO_2$. Run blanks on 15 g of the acid tartrate, using the above volume of $H_2O$ and a soln containing 1 mg of the dye concerned.

(**c**) *For guinea green B, light green SF yellowish, and fast green FCF.*—In a 1-liter Erlenmeyer flask, dissolve 30 g of Na-acid tartrate in 200 cc of $H_2O$; boil to expel air; and under $CO_2$, cool to 85°. Add 50 cc of the dye soln prepared as directed under **47** and 50 cc of $H_2O$, and titrate with the standard $TiCl_3$ soln at 60–70°.

(**d**) *For naphthol yellow S.*—Proceed as directed under (**a**), using as indicator that volume of light green SF yellowish standardized soln (freshly made) that contains 10 mg of dye. Run a blank on the tartrate, light green SF yellowish, and $H_2O$.

### 49 *II. By Precipitation*[29]

*For erythrosine.*—To that volume of a water soln of the sample that contains 0.25 g of dye, add, if necessary, sufficient $H_2O$ to bring the volume to 100 cc. Then add 5 cc of $HNO_3$ of approximately 0.6 *N* strength and filter thru a weighed Gooch crucible. Wash thoroly with 0.5% $HNO_3$ and finally with not more than 10 cc of $H_2O$. The precipitate should not be allowed to cake in the crucible until the washing has been completed. Dry to constant weight at 135°.

## PURE COAL TAR DYE

### 50 *I. By Direct Titration with Standard Titanium Trichloride Solution*

*For yellow AB and yellow OB.*—Dissolve 15 g of Na-acid tartrate in 100 cc of $H_2O$ and add 0.1 g of dye dissolved in 100 cc of 95% alcohol. Titrate with the standard $TiCl_3$ soln, **46**, under $CO_2$, using as an indicator 10 mg of light green SF yellowish from a fresh standardized soln, as directed under **48** (**d**). Run a blank as directed under **48** (**d**), including also the 100 cc of 95% alcohol. 1 cc of 0.1 *N* $TiCl_3 = 0.006180$ g of yellow AB and 0.006530 g of yellow OB. Calculate the percentage of pure dye.

### 51 *II. By Precipitation*

*For erythrosine.*—Multiply the percentage of color acid as obtained under **49** by the factor 1.074.

### 52 *III. By Titration With Potassium Permanganate*

*For indigotine.*—Take that volume of a freshly made water soln of the dye which contains 0.04 g of dye and dilute, if necessary, to 400 cc. Add 2 cc of $H_2SO_4$ and titrate against standard approximately 0.02 *N* $KMnO_4$ soln. The end point is shown by the production of a clear yellow color. The titer of the standard soln must be fixed by titration against a freshly made soln of indigotine of known purity, the same conditions of concentration and acidity being maintained.

### 53 MATTER INSOLUBLE IN CARBON TETRACHLORIDE

*For yellow AB and yellow OB.*—In a 100 cc beaker mix 5 g of the dye with 50 cc of $CCl_4$, stir, and heat to boiling. Wash a Gooch crucible prepared as directed under **28** with $CCl_4$ and heat at 100–105° to constant weight. Filter the hot dye soln thru the crucible, transferring to it the residue in the beaker, and wash with five 10 cc portions of $CCl_4$. Dry at 100–115° and weigh.

### 54 WATER EXTRACTIVES

*For yellow AB and yellow OB.*—(**a**) *Neutral extractive.*—Place 5 g of the well-powdered dye in a 500 cc Erlenmeyer flask or wide-mouthed bottle, add 200 cc of $H_2O$, stopper, and mix by shaking vigorously several times during 2 hours.Filter the mixture, reserving the dye, and evaporate 100 cc of the filtrate on a steam bath in a weighed Pt dish. Dry in an oven at 100–105°, cool, and weigh. The result represents the neutral extractive. Test small portions of the remainder of the filtrate for chlorides, sulfates, and nitrates. If more than traces are present, make the proper analyses on aliquot portions of the filtrate.

(**b**) *Acid extractive.*—Dissolve the residual dye reserved under (**a**) in 100 cc of benzene and, in a separatory funnel, extract with 2 successive 25 cc portions of HCl (1+9). Combine the extracts and wash once with 25 cc of benzene. Draw off the acid extract into a weighed Pt dish. Discard the benzene and wash the funnel with 10 cc of HCl (1+9), adding this washing to the extract in the dish. Evaporate to dryness on the steam bath, dry at 100–105°, cool in a desiccator containing $CaCl_2$, and weigh. The result represents the acid extractive.

### MELTING POINT

*For yellow AB and yellow OB.*—

55

#### APPARATUS

The apparatus, Fig. 21, consists of a tube about 15 cm long and 3.5 cm in internal diameter, with a bulb of 5 cm internal diameter. Fill with glycerin to about the height indicated. Fit the tube with a cork stopper carrying a glass tube (*A*), 5 mm in diameter, which reaches nearly to the bottom of the bath; an ordinary test tube (*B*) in which a thermometer (*C*) is suspended by means of a rubber stopper in such a manner that the Hg column is wholly within the tube and the Hg bulb equidistant from its walls; a long stemmed thermometer (*D*), supported so as to reach a short distance below the tube (*B*); and an outlet tube (*E*) to permit the escape of air and vapor.

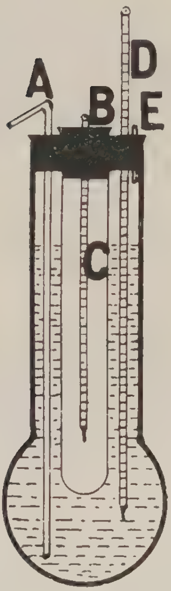

FIG. 21.—MELTING POINT APPARATUS

56

#### DETERMINATION

To a capillary tube of 1 mm or smaller internal diameter, sealed at one end, transfer a small portion of the sample by inserting into the sample the open end of the capillary, removing, inverting, and gently tapping until the well packed substance fills the bottom of the tube to a height of 2–4 mm. Attach the capillary tube to the thermometer *C* by means of a small rubber band, so that the sample is placed at about the middle of the Hg bulb. Replace the thermometer in the tube, connect the tube *A* to the air blast, and force a fairly rapid stream of air bubbles thru the bath. Raise the temp. of the bath rapidly to within 5° of the approximate melting point of the sample. Keep the temp. constant until the thermometer reading is within 1° of that of the bath. Then raise the temp. slowly until the melting point is observed. On approaching within 0.5° of the melting point, the substance darkens; the true melting point is indicated by the formation of a meniscus on the upper surface. When this condition is observed, hold the temp. as nearly constant as possible until the whole of the sample has liquefied.

### LOWER SULFONATED DYES

57

#### REAGENTS

(a) *Titanium trichloride soln.*—0.1 *N*. Prepare and preserve as directed under **46.**

(b) *Salt-acetate soln.*—Dissolve 125 g of NaCl in $H_2O$, add 12 cc of glacial acetic acid and a soln of 13.6 g of Na-acetate, and dilute to 500 cc.

58

#### PREPARATION OF SOLUTION

(a) *For amaranth, tartrazine, ponceau 3R, and indigotine.*—Prepare a water soln of such strength that 50 cc will contain 0.2 g of the dye.

(b) *For light green SF yellowish, and fast green FCF.*—Prepare a water soln of such strength that 10 cc will contain 0.1 g of the dye.

### 59 DETERMINATION

**(a)** *For amaranth and tartrazine.*—To 50 cc of the soln, prepared as directed under **58 (a)**, add 1 cc of HCl. Extract the lower sulfonated dye by shaking the soln successively in 3 separatory funnels, each containing 50 cc of amyl alcohol. Wash the amyl alcohol extracts by shaking successively with three 50 cc portions of 0.25 $N$ HCl, passing each acid portion thru the 3 funnels in the order used for the original soln. (1 volume of HCl, diluted with 50 volumes of $H_2O$, yields a soln of about 0.25 $N$ strength.) Dilute the amyl alcohol in each funnel with 100 cc portions of gasoline (sp. gr. 0.65), and remove the lower sulfonated dye by washing with several 10 cc volumes of $H_2O$, passing each portion thru the 3 funnels in an order the reverse of that previously followed.

Determine the dye in the water extract by titration against the standard $TiCl_3$ soln, using 15 g of Na-acid tartrate, and having a volume of 100 cc. Run a blank determination on all reagents, using 1 mg of the dye concerned. Calculate the result to percentage of fast red E in amaranth and to fast yellow G in tartrazine. 1 cc of 0.1 $N$ $TiCl_3$ soln $=0.01256$ g of fast red E and 0.01081 g of fast yellow G. If the quantity of dye is very low, it may be determined colorimetrically, amaranth or tartrazine, as appropriate, being used as standard.

**(b)** *For ponceau 3R.*—Proceed as directed under **(a)**, substituting a mixture of equal volumes of amyl alcohol and gasoline (sp. gr. 0.65) in place of the amyl alcohol and running the blank with 1 mg of ponceau 3R. Calculate the result to percentage of Na-trimethyl benzene-azo-B-naphthol sulfonate, using the factor 0.009807.

**(c)** *For indigotine.*—Proceed as directed under **(a)**, substituting 0.4 cc of acid for 1 cc, washing with 0.0625 $N$ instead of 0.25 $N$ acid, and running the blank with 1 mg of indigotine. Calculate the result to percentage of Na-indigo monosulfonate, using the factor 0.01821.

**(d)** *For light green SF yellowish and fast green FCF.*—To 10 cc of the dye soln prepared as directed under **58 (b)**, add 40 cc of the salt-acetate soln and extract successively in 3 separatory funnels, each containing 50 cc of amyl alcohol. Wash the extracts with three 50 cc portions of the salt-acetate soln, passing each wash portion successively thru the 3 funnels in the order used for the original extractions. Remove the dye from the alcohol as directed under **(a)** and determine colorimetrically by comparison with a standard guinea green B soln of approximately the same strength for light green SF yellowish, and by comparison with a standard soln of fast green FCF, for the percentage of subsidiary dye. Report as percentage of guinea green B.

### 60 BOILING RANGE OF CUMIDINE FROM PONCEAU 3R

**(a)** Dissolve 60 g of the dye in a 600–700 cc beaker with about 450 cc of boiling $H_2O$, and add the hot soln very slowly to a warm (60–80°) soln of 100 g of $SnCl_2$ in 100 cc of HCl in a tall 1200 cc beaker. Add the dye soln in 10–20 cc portions, waiting after each addition until the mixture is a pale brown; otherwise the dye will be precipitated, in which case it can be reduced only with difficulty. As reduction proceeds and the soln becomes more dilute, heat to boiling, taking care that the mixture does not boil over after each addition of dye, as some heat is generated by the reaction. After all the dye has been added and reduced, allow the mixture to cool, and make alkaline by the addition of about 75 g of NaOH dissolved in 150–200 cc of $H_2O$.

Cool the alkaline mixture and extract the cumidine by shaking it with three 200 cc portions of ether. Combine the ether extracts thus obtained and wash with $H_2O$ until the alkali and salts are removed. Evaporate the solvent on the steam bath, but avoid such prolonged heating as may tend to volatilize the base. Transfer the residue of crude cumidine to a small side-neck flask and distil it, carefully avoiding overheating. Observe the range within which the substance volatilizes.

(b) Proceed as directed under (a) to the directions for the extraction with ether. Then steam distil the alkaline mixture until no more oil is carried over. Separate the oil layer and extract the water layer with two 150 cc portions of ether. Add the extracts to the oil layer and wash the mixture with successive 10 cc portions of $H_2O$ until the alkali and salts are removed. Evaporate the solvent and complete the determination as directed under (a).

### 61 ISOMERIC AND SIMILAR DYES IN AMARANTH

Take a volume of a water soln of the sample that contains 0.1 g of the dye and dilute, if necessary, to 40 cc with $H_2O$. Add 10 cc of 0.1 *N* benzidine soln (9.2 g of base per liter in 0.5 *N* HCl); mix well; and allow to stand exactly 2 min. Filter thru a fluted paper and dilute 10 cc of the filtrate to 100 cc. Compare this soln colorimetrically with a standard amaranth soln containing 0.4 mg of the dye per 100 cc. The soln of the amaranth to be tested may be used in making the standard soln. If, after the benzidine treatment, the soln obtained is not more intensely colored than the standard soln, the proportion of isomeric dyes may be considered to be below 1.5%.

### 62 SODIUM IODIDE

*For erythrosine.*—Dilute to approximately 400 cc that volume of a water soln of the sample that contains 5 g of the dye and add a mixture of 2 cc of $HNO_3$ and 10–20 cc of $H_2O$. Dilute to exactly 500 cc, mix, and filter thru a dry paper. Place 200 cc of the filtrate in a porcelain casserole and make slightly alkaline with 10% NaOH soln. Add approximately 20 cc of 7% $KMnO_4$ soln, mix, and add 10 cc of $HNO_3$. Place on a steam bath and evaporate to dryness. Add 5 cc of 7% $KMnO_4$ and 5 cc of $HNO_3$ and again evaporate to dryness. Then add approximately 50 cc of $H_2O$, 5 cc of $HNO_3$, and 25–30 cc of a saturated soln of $SO_2$. Stir frequently, breaking up any lumps, until the hydrated oxide of Mn has dissolved. Filter, wash the paper with $H_2O$, add to the combined filtrate and washings an excess of 10% $AgNO_3$ soln and boil until the $SO_2$ has been expelled. Collect the precipitate on a weighed Gooch crucible, wash with hot $H_2O$, dry, and weigh. Calculate as percentage of NaI.

### 63 IODINE ORGANICALLY COMBINED

*For erythrosine.*—Place in a porcelain casserole that volume of a water soln of the sample that contains 0.3–0.4 g of the dye. Add 5 cc of a 10% NaOH soln and 35 cc of a 7% soln of pure $KMnO_4$, and mix. Partially cover the vessel with a watch-glass and add 10 cc of $HNO_3$. Place on the steam bath and keep covered until spattering ceases; remove the watch-glass and allow evaporation to proceed to dryness. (Care should be taken to prevent access of reducing gases or vapors to the mixture.) Treat the residue with 5 cc of 7% $KMnO_4$ and 5 cc of $HNO_3$ and again evaporate to dryness. Add approximately 50 cc of $H_2O$, 5 cc of $HNO_3$, and 40 cc of a saturated soln of $SO_2$, and let stand with occasional stirring (breaking up the lumps with a glass rod) until the hydrated oxide of Mn has dissolved.

Filter, wash the paper thoroly with $H_2O$, add an excess of 10% $AgNO_3$ to the combined filtrate and washings, and boil until $SO_2$ has been expelled and the AgI has flocculated. Collect the precipitate on a weighed Gooch crucible, wash with hot $H_2O$, dry, and weigh. Calculate as percentage of free I and from the result subtract the percentage of the I found as NaI, **62**. This result is the I organically combined.

### 64 TOTAL HALOGENS

*For erythrosine.*—Mix 0.5–1 g of the dye with 4 g of $K_2CO_3$ and moisten to a paste with 50% alcohol. Dry, cover with a layer of dry $K_2CO_3$, and ignite at a low red heat. Allow to cool, moisten with a few drops of $H_2O$, and break up the charred mass thoroly. Wash into a beaker with about 20 cc of $H_2O$, allow to digest for 15 min., and filter. Wash the insoluble matter until the washings no longer react with $AgNO_3$; then acidify the filtrate and washings with $HNO_3$, using an excess equivalent to 5 cc of the strong acid and precipitate the halogens with 10% $AgNO_3$ soln. Collect the precipitate on a weighed Gooch crucible, wash, dry, and weigh. Compare with the sum of the results obtained in the separate halogen determinations.

### 65 SODIUM CARBONATE

*For erythrosine.*—Determine total $CO_2$ as directed under **XV, 4,** using a 10 g sample. Calculate and report as $Na_2CO_3$.

### 66 ORANGE II IN ORANGE I

To a volume of a water soln of the sample that contains 1 g of dye add $H_2O$, if necessary, to bring the volume to 100 cc, and 10 cc of HCl. Extract this soln by shaking successively in three 500 cc separatory funnels, each containing 100 cc of amyl alcohol. Wash each of the 3 amyl alcohol extracts by means of six 100 cc portions of $N$ $Na_2CO_3$ soln (53 g of anhydrous $Na_2CO_3$ to the liter), passed successively thru the funnels in the order first used. (In washing the acidified amyl alcohol solns, shake gently at first, keeping the funnel upright and unstoppered until the evolution of $CO_2$ is slow enough to permit more vigorous shaking.) In the same manner wash the extracts in the second and third funnels with 2 more 100 cc portions of the $Na_2CO_3$ soln and wash the extract in the third funnel with 2 additional portions of the carbonate soln. Dilute the amyl alcohol solns by adding 350 cc of gasoline (sp. gr. 0.65) to each funnel. Remove the dye by extracting completely with the requisite number of 10 cc portions of $H_2O$ passed thru the funnels, reversing the order previously used. Bring the volume to 100 or 150 cc by adding $H_2O$, add about 10 g of Na-acid tartrate, and titrate with standard $TiCl_3$ soln, **46.** 1 cc of 0.1 $N$ $TiCl_3 =$ 0.008756 g of orange II.

### 67 MARTIUS YELLOW IN NAPHTHOL YELLOW S

Dissolve 5 g of the dye in 150 cc of $H_2O$, add 5 cc of HCl, and shake vigorously in a separatory funnel for 1 min. with 50 cc of gasoline (sp. gr. 0.65). Separate the solns and extract the aqueous liquid again with 25–30 cc of the solvent. Combine the portions of gasoline, decant into a clean separatory funnel, and wash with four 25 cc portions of 0.25 $N$ HCl. Remove the martius yellow by shaking with a few portions of 5% NaOH soln. Neutralize the alkaline dye soln with tartaric acid, add Na-tartrate, if necessary, and titrate against standard $TiCl_3$ soln as directed under **48 (d).** 1 cc 0.1 $N$ $TiCl_3 = 0.002134$ g of martius yellow.

Very small quantities (less than 0.1%) may also be determined colorimetrically (in neutral or slightly alkaline soln) by comparison with a standard naphthol yellow S soln, the tinctorial power of which is considered to be 8/10 that of martius yellow.

### 68 TOTAL NITROGEN

**(a)** *For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF, indigotine, yellow AB, and yellow OB.*—Proceed as directed by Dumas.[27]

(b) *For guinea green B, light green SF yellowish, fast green FCF, and indigotine.*—Proceed as directed under **II, 22,** using 2 g of the sample and a little $CuSO_4$ to assist the oxidation.

(c) *For ponceau 3R, amaranth, orange I, and tartrazine.*—Treat 2 g of the dye with 25 cc of a saturated soln of $SO_2$ and 1 g of Zn dust, and warm the mixture gently until it becomes colorless. (This should take place in 2-3 min., but if it does not, add more $SO_2$ soln in small portions at a time until the color is destroyed.) Then add 30 cc of $H_2SO_4$ and 0.7 g of HgO or its equivalent of metallic Hg and digest the mixture. Finally make alkaline, distil, and titrate as directed under **II, 19, 22** or **24.**

**69** *Percentage of sulfur, nitrogen and sodium in permitted food dyes*

| DYE | SULFUR | NITROGEN | SODIUM | SODIUM SULFATE CORRESPONDING TO SODIUM CONTENT |
|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* |
| Ponceau 3R | 12.97 | 5.66 | 9.30 | 28.71 |
| Amaranth | 15.91 | 4.64 | 11.41 | 35.23 |
| Erythrosine | ..... | ..... | 5.12 | 15.81 |
| Orange I | 9.15 | 8.00 | 6.57 | 20.28 |
| Naphthol yellow S | 8.95 | 7.82 | 12.84 | 39.64 |
| Tartrazine: | | | | |
| Trisodium salt | 12.00 | 10.49 | 12.91 | 39.85 |
| Disodium salt | 12.52 | 10.94 | 8.98 | 27.72 |
| Guinea green B[1] | 9.29 | 4.06 | 3.33 | 10.28 |
| Light green SF yellowish:[1] | | | | |
| Disodium salt | 12.13 | 3.53 | 5.80 | 17.90 |
| Monosodium salt | 12.48 | 3.64 | 2.98 | 9.20 |
| Fast green FCF | 11.89 | 3.46 | 5.69 | 17.57 |
| Indigotine | 13.75 | 6.01 | 9.86 | 30.42 |
| Yellow AB | ..... | 17.00 | ..... | ..... |
| Yellow OB | ..... | 16.09 | ..... | ..... |

[1] There is evidence that the disodium salt of guinea green B and the trisodium salt of light green SF yellowish form colorless solutions.

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# XXII. DAIRY PRODUCTS

## MILK

### 1 COLLECTION OF SAMPLE—OFFICIAL

The quantity of sample required depends upon the number of determinations to be made. For the usual analysis collect 250–500 cc (½ pint) of sample; for the fat determination only, 50–60 cc (approximately 2 fl. oz.) will suffice.

In the case of bottled milk collect one or more bottles as prepared for sale. In sampling bulk milk thoroly mix by pouring from one clean vessel into another 3 or 4 times. If this procedure is impracticable, thoroly stir the milk for at least 30 seconds with a suitable appliance long enough to reach to the bottom of the container. If cream has formed on the milk, continue the mixing until all cream is detached from the sides of the vessel and evenly emulsified thruout the liquid.

Place the samples in non-absorbent, air-tight containers and keep them in the cold, but at a temp. above freezing, until ready for examination. When transported by mail, express, or otherwise, completely fill the containers, tightly stopper, and mark for identification. A suitable quantity of preservative ($HgCl_2$, $K_2Cr_2O_7$, or $HCHO$) may be used unless the presence of the preservative is objectionable in connection with physical or chemical tests to be applied in addition to the determination of fat.

### 2 PREPARATION OF SAMPLE—OFFICIAL

Before withdrawing portions for analytical determinations, bring the sample to a temp. of 15–20° and mix thoroly by pouring into a clean receptacle and back until a homogeneous mixture is assured. If lumps of cream do not completely disappear, warm the sample to about 38°, mix thoroly, then cool to 15–20°. In case a measured volume is required in a determination, bring the temp. of the sample to 20° before pipetting.

### 3 SPECIFIC GRAVITY—TENTATIVE

Determine specific gravity at 15.6/15.6° by means of a pycnometer (cf. **XVII, 24**, or by means of a standardized hydrometer.

### 4 ACIDITY—TENTATIVE

Dilute 10–20 cc of the milk with an equal volume of recently boiled and cooled $H_2O$ and titrate with standard NaOH soln, using phenolphthalein indicator. Express the result as percentage of lactic acid. The determination may be conveniently made by measuring 17.6 cc of the prepared sample with the 17.6 cc Babcock pipet [**18 (b)**], diluting with an equal volume of recently boiled and cooled $H_2O$, washing out the pipet with $CO_2$-free $H_2O$, and titrating with 0.1 $N$ NaOH soln, using 0.5 cc of phenolphthalein indicator. The number of cc of 0.1 $N$ NaOH soln required divided by 20 gives the percentage of lactic acid.

### 5 TOTAL SOLIDS—OFFICIAL

Weigh a flat-bottomed dish of not less than 5 cm diameter. If desired, the dish may have spread in it, prior to weighing, 15–20 g of pure dry sand. Pipet into the dish 3–5 cc of the sample, weigh quickly, and heat at the temp. of boiling $H_2O$ until it ceases to lose weight. Cool in a desiccator and then weigh quickly to avoid the absorption of moisture. Report the increase in weight as total solids.

### 6 ASH—OFFICIAL

Into a weighed dish pipet about 20 cc of the prepared sample, weigh quickly, add 6 cc of $HNO_3$, evaporate to dryness, and ignite at a temp. below redness until the ash is free from C. Cool in a desiccator, weigh, and report the increase in weight as ash.

### 7 TOTAL NITROGEN—OFFICIAL

Transfer 5 g of the sample to a Kjeldahl digestion flask and proceed as directed under **II, 19, 22,** or **24.** Multiply the percentage of N by 6.38 to obtain the equivalent percentage of N compounds.

## CASEIN

(This determination should be made while the milk is fresh, or nearly so. When it is not practicable to make this determination within 24 hours, add 1 part of HCHO to 2500 parts of milk and keep in a cool place.)

### 8 *Method I—Official*

Place 10 g of the sample in a beaker with 90 cc of $H_2O$ at 40–42°, and add at once 1.5 cc of acetic acid (1+9). Stir, and let stand 3–5 min. Decant on a filter, wash by decantation 2 or 3 times with cold $H_2O$, and transfer the precipitate to the filter. Wash once or twice on the filter. The filtrate should be clear, or very nearly so. If the first portions of the filtrate are not clear, repeat the filtration, and complete the washing of the precipitate. Determine N in the washed precipitate and filter paper as directed under **II, 19, 22** or **24,** and multiply by 6.38 to obtain the equivalent of casein.

To a sample of milk that has been preserved, the acetic acid should be added in small portions, a few drops at a time, with stirring, and the addition should be continued until the liquid above the precipitate becomes clear, or very nearly so.

### *Method II[1]—Tentative*

#### REAGENT

Pipet 250 cc of *N* acetic acid into a 1000 cc flask. Add 125 cc of normal $CO_2$-free NaOH. Make up to 1000 cc with $CO_2$-free distilled $H_2O$ and mix thoroly.

#### 9 DETERMINATION

Pipet 20 cc of the sample into a 100 cc flask. Add 50 cc of the reagent, mix, make up to volume with distilled $H_2O$, and shake well. Set the flask in hot $H_2O$ (50–60°, *not over* 60°) and let stand 15 min. Cool to room temp., add 0.5 g of filter-cell, shake thoroly, and filter clear thru a suitable folded paper, taking care to prevent evaporation during filtration. Determine N (A) in 50 cc of the clear filtrate, and determine total N (B) in 10 cc of the milk. Multiply (B−A) by 6.38. This gives the casein in 10 cc of the milk. Report grams of casein per 100 cc of milk, or divide the grams per 100 cc by the density of the milk and report as percentage by weight.

## ALBUMIN

### 10 *Method I—Official*

Exactly neutralize the filtrate obtained under **8** with 10% NaOH soln, add 0.3 cc of acetic acid (1+9), and heat on a steam bath until the albumin is completely precipitated. Collect the precipitate on a filter; wash with cold $H_2O$; determine the N as directed under **II, 19, 22** or **24**; and multiply by 6.38 to obtain the equivalent of albumin.

### 11 *Method II—Official**

## LACTOSE

### *Optical Method—Official*

### 12 REAGENTS

(a) *Acid mercuric nitrate soln.*—Dissolve Hg in twice its weight of $HNO_3$ and dilute with an equal volume of $H_2O$.

(b) *Mercuric iodide soln.*—Dissolve 33.2 g of KI and 13.5 g of $HgCl_2$ in 20 cc of glacial acetic acid and 640 cc of $H_2O$.

### 13 DETERMINATION

Determine the specific gravity of the milk as directed under **3.** The quantity of sample to be taken for the determination varies with the specific gravity and is to be measured at the same temp. at which the specific gravity is taken. The volume to be measured will be found in the table under **14,** which is based upon twice the normal weight of lactose (32.9 g per 100 cc) for the Ventzke sugar scale.

Place the quantity of milk indicated under **14** in a flask graduated at 102.6 cc. Add 1 cc of the acid $Hg(NO_3)_2$ soln or 30 cc of the $HgI_2$ soln (an excess of these reagents does no harm), fill to the mark, shake, filter thru a dry filter, and polarize. It is not necessary to heat before polarizing. If a 200 mm tube is used, divide the polariscope reading by 2 (or, if a 400 mm tube is used, by 4) to obtain the percentage of lactose in the sample.

**14** *Volumes of milk corresponding to a lactose double normal weight*[2]

| SPECIFIC GRAVITY OF MILK | VOLUME OF MILK FOR A LACTOSE DOUBLE NORMAL WEIGHT (VENTZKE SCALE) | SPECIFIC GRAVITY OF MILK | VOLUME OF MILK FOR A LACTOSE DOUBLE NORMAL WEIGHT (VENTZKE SCALE) |
|---|---|---|---|
| 1.024 | 64.25 | 1.030 | 63.90 |
| 1.025 | 64.20 | 1.031 | 63.80 |
| 1.026 | 64.15 | 1.032 | 63.75 |
| 1.027 | 64.05 | 1.033 | 63.70 |
| 1.028 | 64.00 | 1.034 | 63.65 |
| 1.029 | 63.95 | 1.035 | 63.55 |
| | | 1.036 | 63.50 |

### 15 *Gravimetric Method—Official*

Dilute 25 g of the sample with 400 cc of $H_2O$ in a 500 cc volumetric flask and add 10 cc of $CuSO_4$ soln [**XXXIV, 31** (a)] and about 7.5 cc of a KOH soln of such strength that 1 volume is just sufficient to precipitate completely the Cu as hydroxide from 1 volume of the $CuSO_4$ soln. Instead of KOH soln of this strength, 8.8 cc of 0.5 *N* NaOH soln may be used. After the addition of the alkali soln, the mixture must still have an acid reaction and contain Cu in soln. Fill the flask to the 500 cc mark, mix, filter thru a dry filter, and determine lactose in an aliquot of the filtrate as directed under **XXXIV, 54** or **56.**

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* See note 7, p. xvii.

### FAT

### 16 *Roese-Gottlieb Method*[3]*—Official*

Transfer 10 g of the sample to a Röhrig tube or a similar apparatus, add 1.25 cc of $NH_4OH$ (2 cc if the sample is sour), and mix thoroly. Add 10 cc of 95% alcohol and mix well. Add 25 cc of ether, shake vigorously for 30 seconds, add 25 cc of petroleum ether (redistilled slowly at a temp. below 65°), and shake again for 30 seconds. Let stand 20 min., or until the upper liquid is practically clear. Draw off as much as possible of the ether-fat soln (usually 0.5–0.8 cc will be left) into a flask thru a small, quick-acting filter. Again extract the liquid remaining in the tube, this time with 15 cc of each ether; shake vigorously 30 seconds after each addition; and allow to settle. Draw off the clear soln thru the small filter into the same flask as before and wash the tip of the spigot, the funnel, and the filter with a few cc of a mixture of the two ethers, in equal parts, free from suspended $H_2O$. To insure complete removal of the fat, a third extraction is necessary. This third extraction yields less than 1 mg of fat if the previous ether-fat solns have been drawn off closely. Add a glass bead and evaporate the ethers slowly on a steam bath; then dry the fat in a boiling $H_2O$ oven to constant weight. Weigh the flask with a similar flask as a counterpoise. Do not wipe the flask immediately before weighing.

Remove the fat completely with petroleum ether. Deduct the weight of the dried flask with residue and bead to obtain weight of fat. Finally, correct this weight by a blank determination on the reagents used.

### *Babcock Method*[4]*—Official*

### 17 REAGENT

*Sulfuric acid.*—Sp. gr. 1.82–1.83 at 20°.

### 18 APPARATUS

(a) *Standard Babcock test bottle for milk.*—8%, 18-g, 6-inch milk-test bottle, total height 150–165 mm (5.9–6.5 inches). The bottom of the bottle shall be flat, and the axis of the neck shall be vertical when the bottle stands on a level surface. The charge of milk for the bottle shall be 18 g.

*Bulb.*—The capacity of the bulb to the junction with the neck shall be not less than 45 cc. The shape of the bulb shall be either cylindrical or conical. If cylindrical, the outside diameter shall be between 34 and 36 mm; if conical, the outside diameter of the base shall be between 31 and 33 mm, and the maximum diameter between 35 and 37 mm.

*Neck.*—The neck shall be cylindrical and of uniform diameter from at least 5 mm below the lowest graduation mark to at least 5 mm above the highest. The top of the neck shall be flared to a diameter of not less than 10 mm. The graduated portion of the neck shall have a length of not less than 63.5 mm. The total per cent graduation shall be 8. The graduations shall represent whole per cent, 0.5%, and 0.1%, respectively, from 0.0 to 8.0%. The tenths per cent graduations shall be not less than 3 mm in length; the 0.5% graduations shall be not less than 4 mm in length and shall project 1 mm to the left; and the whole per cent graduations shall extend at least half-way around the neck to the right and shall project at least 2 mm to the left of the tenths per cent graduations. Each whole per cent graduation shall be numbered, the number being placed to the left of the scale. The capacity of the neck for each whole per cent on the scale shall be 0.20 cc. The maximum error of the total graduation or any part thereof shall not exceed the volume of the smallest unit of the graduation.

Each bottle shall be so constructed as to withstand the stress to which it will be subjected in the centrifuge.

($a_1$) *Testing.*—The Hg and cork, alcohol and buret, and alcohol and brass plunger methods may be employed for the rapid testing of the bottles, but the accuracy of any questionable bottle shall be determined by calibration with Hg (13.5471 g of clean, dry Hg at 20° to be equal to 5% on the scale of an 18-g bottle and 10% on the scale of a 9-g bottle), the bottle being previously filled to zero with Hg.

(b) *Pipet.*—The standard milk pipet shall conform to the following specifications:

*Total length:* not more than 330 mm.
*Outside diameter of suction tube:* 6–8 mm.
*Length of suction tube:* 130 mm.
*Outside diameter of delivery tube:* 4.5–5.5 mm.
*Length of delivery tube:* 100–120 mm.
*Distance of graduation mark above bulb:* 15–45 mm.
*Nozzle:* straight.
*Graduation:* to contain 17.6 cc of $H_2O$ at 20°, when the bottom of the meniscus coincides with the mark on the suction tube.
Delivery in 5–8 seconds.
The maximum error in the graduation shall not exceed 0.05 cc.
The pipet is to be marked "Holds 17.6 cc."

($b_1$) *Testing.*—The pipet shall be tested by measuring from a buret the volume of $H_2O$ (at 20°) which it holds up to the graduation mark.

(c) *Acid measure.*—The device employed to measure $H_2SO_4$, whether a graduated cylinder or a pipet attached to a Swedish acid bottle, shall be graduated to deliver 17.5 cc.

(d) *Centrifuge or "tester."*—The standard centrifuge, however driven, shall be constructed thruout and so mounted as to be capable, when filled to capacity, of rotating at the necessary speed with a minimum of vibration and without liability of causing injury or accident. It shall be heated, electrically or otherwise, to a temp. of at least 55° during the process of centrifugalizing. It shall be provided with a speed indicator, permanently attached, if possible. The proper rate of rotation may be ascertained by reference to the table below. By "diameter of wheel" is meant the distance between the inside bottoms of opposite cups measured thru the center of rotation of the centrifuge wheel while the cups are horizontally extended.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Diameter of wheel, in inches: | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 |
| No. revolutions per minute: | 1074 | 980 | 909 | 848 | 800 | 759 | 724 | 693 |

(e) *Dividers or calipers.*—For measuring the fat column.

(f) *Water bath for test bottles.*—Provided with a thermometer and a device for maintaining a temp. of 55–60°.

### 19 DETERMINATION

Transfer 18 g of the sample, prepared as directed under **2**, to the milk-test bottle by means of the pipet. Blow out the milk remaining in the pipet tip after free outflow has ceased. Add 17.5 cc of $H_2SO_4$, preferably not all at one time, pouring it down the side of the neck of the bottle in such a way as to wash any traces of the milk into the bulb. The temp. of the acid shall be about 15–20°. Shake until all traces of curd have disappeared; then transfer the bottle to the centrifuge; counterbalance it; and, after the proper speed has been attained, whirl 5 min. Add soft $H_2O$ at 60°, or above, until the bulb of the bottle is filled. Whirl 2 min. Add hot $H_2O$ until the liquid column approaches the top graduation of the scale. Whirl 1 min. longer at a temp. of 55–60°. Tansfer the bottle to the warm water bath maintained at a temp. of 55–60°, immerse it to the level of the top of the fat column, and leave it there until the column is in equilibrium and the lower fat surface has assumed a final form. Remove the bottle from the bath; wipe it; and, with the aid of dividers or calipers, measure the fat column, in terms of percentage by weight, from its lower surface to the highest point of the upper meniscus.

The fat column, at the time of measurement, should be translucent, of a golden yellow or amber color, and free from visible suspended particles. Reject all tests in which the fat column is milky or shows the presence of curd or of charred matter, or in which the reading is indistinct or uncertain.

## ADDED WATER

**20**

### *I. Acetic Serum Method—Official*

(**a**) *Zeiss immersion refractometer reading.*—To 100 cc of the milk, measured at 20° into a beaker, add 2 cc of 25% acetic acid (sp. gr. 1.035). Cover the beaker with a watch-glass and place in a water bath at 70° for 20 min. Place the beaker in ice $H_2O$ for 10 min. and separate the curd from the serum by rapid filtration thru a small filter. Transfer a portion of the clear serum to a refractometer beaker, place in the constant temp. bath, and take the refractometer reading when the temp. of the serum has been brought to exactly 20°, as determined by a thermometer graduated in tenths of a degree. A reading below 39 indicates added $H_2O$; between 39 and 40, the addition of $H_2O$ is suspected. When the reading is 40 or below, determine the ash in the serum as directed under (**b**).

(**b**) *Ash.*—Transfer 25 cc of the serum to a weighed flat-bottomed Pt dish and evaporate to dryness on a water bath. Heat over a low flame (to avoid spattering) until the contents are thoroly charred, place the dish in an electric muffle, preferably with pyrometer attached, and ignite to a white ash at a temp. not greater than 500°. Cool and weigh. Express the result as grams per 100 cc. A result below 0.715 g per 100 cc indicates added $H_2O$. The acetic serum ash, multiplied by the factor 1.021, equals the sour serum ash (dilution of the acetic serum being 2%).

**21**

### *II. Sour Serum Method—Official*

(**a**) *Zeiss immersion refractometer reading.*[6]—Allow the milk to become completely sour, filter, and determine the immersion refractometer reading of the clear serum at 20°. A reading below 38.3 indicates added $H_2O$.

(**b**) *Ash.*[7]—Determine the ash in 25 cc of the serum, obtained in (**a**), as directed under **20** (**b**). A result below 0.730 g per 100 cc indicates added $H_2O$.

**22**

### *III. Copper Serum Method[8]—Official*

To 1 volume of $CuSO_4$ soln (72.5 g of $CuSO_4.5H_2O$ per liter, adjusted if necessary to read 36 at 20° on the scale of the Zeiss immersion refractometer, or to a specific gravity of 1.0443 at 20/4°), add 4 volumes of milk. Shake well and filter. Determine the refractometer reading of the clear serum at 20°. A reading below 36 indicates added $H_2O$. When the refractometer reading is 36 or below, determine the ash of the sour serum as directed under **21** (**b**) or of the acetic serum as directed under **20** (**b**).

### *IV. Cryoscopic Method[9]—Official*

**23**

#### APPARATUS

(**a**) *Cryoscope.*—A cylindrical-shaped Dewar flask of 1 liter capacity and 28 cm internal depth, surrounded by a metal casing, is tightly closed by means of a large cork of about 3 cm thickness. Thru the center of the cork is tightly fitted a medium thin-walled glass or metal tube, 250 mm in length by 33 mm outside diameter. At one side of the cork is inserted a narrow metal inlet tube, the lower end of which is formed into a perforated loop near the bottom of the flask. At the opposite side is a metal tube of T-shape construction and 6 mm internal diameter, intended to afford escape for vapors, and also for introducing volatile fluid into the apparatus. At the back portion of the cork is fitted a control thermometer, the bulb of which extends nearly to the bottom of the flask. The freezing test tube is of thin glass, about 240 mm in length by 29 mm outside diameter, and fits closely into the larger tube, which is sealed into the cork. In the rubber stopper of the freezing tube is fitted the standard thermometer. The length of the thermometer permits insertion of the bulb nearly to the bottom of the tube and at the same time allows complete exposure of the scale above the stopper. At the right side of the thermometer a stirring device made of non-corrodible low conductivity metal is fitted into the stopper thru a short section of thin-walled metal tubing. The lower end extends nearly to the bottom of the test tube and is provided with a horizontal loop encircling the thermometer. At the left of the thermometer is a freezing-starter attachment inserted thru an opening in the stopper formed by means of a short section of metal tubing. This device consists of a non-corrodible metal rod, at the lower end of which is a 10 mm length opening for the purpose of carrying a small fragment of ice. At one side of the cryoscope is installed an air-drying arrangement which consists of a Folin absorption bulb inserted thru a tightly fitting stopper and extending nearly to the bottom of a large-sized test tube. A short section of glass tubing is inserted thru a second opening in the stopper and is connected to the vaporizing tube which enters the cryoscope. Sulfuric acid is poured into the drying tube to a level slightly above the small inner bulb. At the opposite side of the apparatus is arranged a drain tube for the purpose of conducting vapors away from the operator. By means of a pressure and suction pump dry air may be forced into the apparatus at a suitable rate and the mixed vapors conducted out thru the base of the drain tube into the sink. An adjustable lens is mounted in a convenient position in front of the thermometer for the purpose of magnifying the scale.

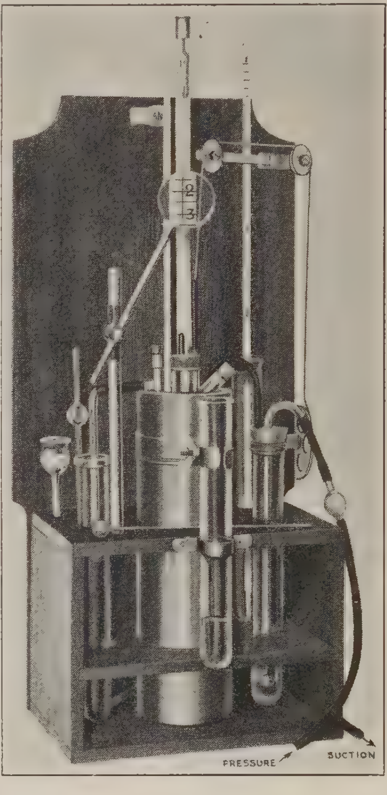

FIG. 22.—HORTVET CRYOSCOPE

(b) *Standard thermometer.*—A solid-stem instrument having a total length of 58 cm, with a scale portion measuring about 30 cm. The total scale range is 3°, from +1° to −2°, and each degree division is subdivided into tenths and hundredths. The length of a degree division approximates 1 dm, thus making the smallest subdivisions of such magnitudes as to enable easy observation and readings estimated to 0.001°. Standardize the thermometer as directed under **24**. Check at frequent intervals, once a week or as often as may be necessary, to keep an accurate record of any changes that may occur.

(**c**) *Control thermometer.*—A solid-stem instrument approximately 58 cm in length and having a scale range of +20° to −30°. Test in a bath of melting crushed ice for the purpose of determining whether the 0-mark on the scale is correct. The scale graduations should be accurate to within 0.10°.

## **24** STANDARDIZATION OF THE THERMOMETER

Make 3 freezing-point determinations by the procedure given under **25** on each of the following:

(**a**) *Recently boiled distilled water.*

(**b**) *Sucrose soln.*—Dissolve 7 g of pure sucrose in $H_2O$ and make the soln to a volume of 100 cc at 20°

(**c**) *Sucrose soln.*—Dissolve 10 g of pure sucrose in $H_2O$ and make the soln to a volume of 100 cc at 20°.

(A sample of pure sucrose may be obtained by application to the Director of the Bureau of Standards, Department of Commerce, Washington, D. C.)

Tabulate the results in the following form:

| FREEZING-POINT OBSERVATIONS | PURE WATER | 7 GRAMS SUCROSE SOLUTION | | 10 GRAMS SUCROSE SOLUTION | |
|---|---|---|---|---|---|
| | | Observed freezing point (−S) | Freezing-point depression S—W (algebraic) | Observed freezing point (−S) | Freezing-point depression S—W (algebraic) |
| 1st | | | | | |
| 2nd | | | | | |
| 3rd | | | | | |
| Averages | ± W | xxxxxxx | | xxxxxxx | |

Express the results as degrees freezing-point depression below the average of the observed freezing points obtained on the sample of pure $H_2O$ (± W), which may be above (+) or below (−) the 0-mark on the scale. Obtain each freezing-point depression of the sucrose solns by the algebraic subtraction of the average of the freezing-point readings of pure $H_2O$ (± W) from each observed freezing point.

Omit adventitious results, *i.e.*, results which are in marked disagreement with other results obtained by carefully following instructions.

Apply the average of the freezing-point depressions obtained on the standard sucrose solutions for the purpose of correcting the thermometer readings obtained on samples of milk in the manner illustrated in the tables accompanying Fig. 23.

## **25** DETERMINATION

(Make freezing-point determinations only on samples of milk that show an acidity of not more than 0.18% when determined as directed under **4**.)

Insert the funnel-tube into the vertical portion of the T-tube at one side of the apparatus and pour in 400 cc of ether previously cooled to 10° or lower. Close the vertical tube by means of a small cork and connect the pressure pump to the inlet tube of the air-drying attachment. Adjust the pump so as to pass air thru the apparatus at a moderate rate, as may be judged by the agitation of the $H_2SO_4$ in the drying tube. Continuous vaporization of the ether will cause a lowering of the temp.

in the flask, from ordinary room temp. to 0° in from 5 to 10 min. Continue the temp. lowering until the control thermometer registers near −3°. At this stage, by lowering the gage tube into the ether bath, then closing the top by means of the forefinger and raising to a suitable height, an estimate can be made as to the quantity of ether necessary to pour in for the purpose of restoring the 400 cc volume. When the volume of ether has been adjusted to 400 cc an additional 10–15 cc is sufficient on an average for each succeeding determination. Pour into the freezing test tube sufficient $H_2O$ (30–35 cc), boiled and cooled to 10° or lower, to fairly submerge the thermometer bulb. Insert the thermometer together with the stirrer and lower the test tube

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Two Bureau of Standards tested thermometers give intervals of 0.199° and 0.200°, respectively, between the freezing-point depression readings of the two sucrose solns. One thermometer gives freezing-point depressions −0.422° and −0.622°, respectively, for the two sucrose solns, while the other gives −0.422° and −0.621°, respectively.

Laboratory Thermometer No. 2.

| WATER | 7 GRAMS SUCROSE TO 100 CC | 10 GRAMS SUCROSE TO 100 CC |
|---|---|---|
| +0.056° | −0.425° | −0.621° |

Interval = 0.196
0.196 equiv. 0.199
Correction = ×1.015

Laboratory Thermometer No. 24.

| WATER | 7 GRAMS SUCROSE TO 100 CC | 10 GRAMS SUCROSE TO 100 CC |
|---|---|---|
| 0.000 | −0.420° | −0.625° |

Interval = 0.205
0.205 equiv. 0.199
Correction = ×0.971

*Example*:

Laboratory Thermometer No. 24.
F. pt. Depression Sample Milk = 0.548
(0.548 − 0.420) 0.971 = 0.124
Corrected Depression = 0.422 + 0.124
= 0.546

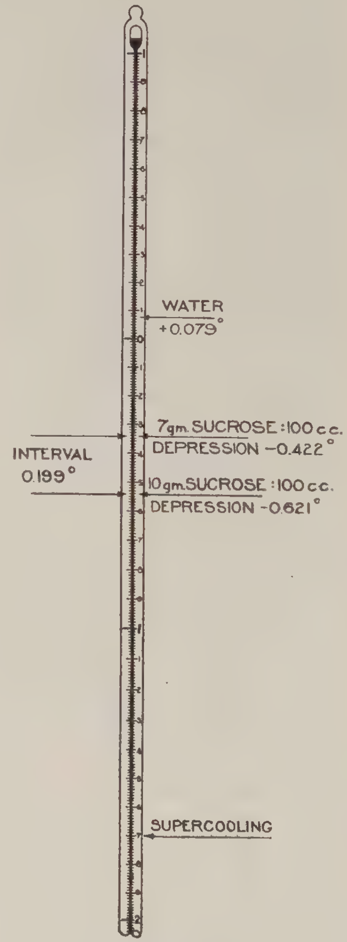

FIG. 23.—U. S. BUREAU OF STANDARDS TESTED THERMOMETER

into the larger tube. A small quantity of alcohol, sufficient to fill the lower space between the 2 test tubes, will serve to complete the conduction medium between the freezing bath and the liquid to be tested. Keep the stirrer in steady up-and-down motion at a rate of approximately one stroke each 1 or 2 seconds, or even at a slower rate, providing the cooling proceeds satisfactorily. Maintain a passage of air thru the apparatus until the temp. of the cooling-bath reaches −2.5°, at which time the top of the Hg thread in the thermometer usually recedes to a position near the freezing point of $H_2O$. Maintain the temp. of the cooling-bath at −2.5° and continue the manipulation of the stirrer until a super-cooling of sample of 1.0 to 1.2° is observed. As a rule, at this time the liquid will begin to freeze, as may be noted by the rapid rise of the Hg. Manipulate the stirrer slowly and carefully 3 or 4 times as the Hg column approaches its highest point. By means of a suitable light-weight cork mallet tap the upper end of the thermometer cautiously a number of times until the top of the Hg column remains stationary for at least 1 min. Observe the exact reading on the thermometer scale, taking necessary precautions to avoid parallax, and estimate to 0.001°. When the observation has been satisfactorily completed, make a duplicate determination; then remove the thermometer and stirrer and empty the $H_2O$ from the freezing tube.

Rinse the tube with about 25 cc of the sample of milk, cooled to 10° or lower; measure into the tube 30–35 cc of milk, or enough to fairly submerge the thermometer bulb; and insert the tube into the apparatus. Maintain the temp. of the cooling-bath at 2.5° below the probable freezing point of the sample. Make the determination on the milk, following the same procedure as that employed in determining the freezing point of $H_2O$. As a rule, however, it is necessary to start the freezing action in the milk by inserting the freezing starter (which has been kept in contact with ice for several minutes, and in the open end of which has been wedged a fragment of ice) at the time when the Hg column has receded to 1.0-1.2° below the probable freezing point. A rapid rise of the Hg results almost immediately. Manipulate the stirrer slowly and carefully 2 or 3 times while the Hg approaches its highest point. Complete the adjustment of the Hg column in the same manner as in the preceding determination; then, avoiding parallax, observe the exact reading on the thermometer scale and estimate to 0.001°. *The algebraic difference* between the average of readings obtained on the $H_2O$ and the reading obtained on the sample of milk represents the *freezing-point depression* of the milk. Apply necessary correction to the result in the manner shown in the illustrative tables accompanying Fig. 23.

Ascertain the percentage of added $H_2O$ corresponding to the determined freezing-point depression from Table **23** under **XLII.** The percentage of added $H_2O$ ($W$) may also be calculated as follows:

$$W = \frac{100\,(T - T^1)}{T}, \text{ in which}$$

$T$ = the average freezing point of normal milk (−0.550°), and
$T^1$ = the observed freezing point on a given sample.

A tolerance of 3% may be allowed on results for added $H_2O$ determined on the basis of an average freezing-point depression of −0.550°. Owing to the narrow variations found in market milk of genuine character it is not necessary to deduct the tolerance figure from results showing added $H_2O$ in excess of 3%.

## GELATIN

### 26 *Qualitative Test—Official*

To 10 cc of the milk add an equal volume of acid $Hg(NO_3)_2$ soln. (Hg dissolved in twice its weight of $HNO_3$ and this soln diluted to 25 times its volume with $H_2O$), shake the mixture, add 20 cc of $H_2O$, shake again, allow to stand 5 min., and filter. If much gelatin is present, the filtrate will be opalescent and cannot be obtained quite clear. To a portion of the filtrate contained in a test tube add an equal volume of saturated aqueous picric acid soln. A yellow precipitate will be produced in the presence of any considerable quantity of gelatin, while smaller quantities will be indicated by a cloudiness. In the absence of gelatin the filtrate will remain perfectly clear.

### 27 PRESERVATIVES—OFFICIAL

Proceed as directed under **XXXII.** To test for salicyclic acid or benzoic acid acidify 100 cc of the milk with 5 cc of HCl (1+3), shake until curdled, filter, and treat the clear filtrate as directed under **XXXII, 2, 3** or **6.**

To test for HCHO proceed as directed under **XXXII, 18–24,** inclusive, applying the test directly to the milk.

### 28 COLORING MATTERS[10]—OFFICIAL

Warm about 150 cc of milk in a casserole over a flame and add about 5 cc of acetic acid (1+3), then slowly continue the heating nearly to the boiling point while stirring. Gather the curd, when possible, into one mass with a stirring rod and pour off the whey. If the curd breaks up into small flecks, separate from the whey by straining thru a sieve or colander. Press the curd free from adhering liquid; transfer to a small flask; and macerate for several hours, preferably overnight, with about 50 cc of ether, keeping the flask tightly corked and shaking at intervals. Decant the ether extract into an evaporating dish, remove the ether by evaporation, and test the fatty residue for annatto as directed under **XXI, 21.**

The curd of an uncolored milk is perfectly white after complete extraction with ether, as is also that of a milk colored with annatto. If the extracted fat-free curd is distinctly orange or yellowish in color, a coal tar dye is indicated. In many cases if a lump of a fat-free curd in a test tube is treated with a little HCl the color changes to pink, indicating the presence of a dye similar to aniline yellow or butter yellow or perhaps one of the acid azo yellows or oranges. In such cases, separate and identify the coloring matter present in the curd as directed under **XXI, 12.** If aniline yellow, butter yellow, or any other oil-soluble dye is present, the greater part will be found in the ether extract containing the fat. In such cases proceed as directed under **XXI, 3.**

In some cases the presence of coal tar dyes can be detected by treating about 100 cc of the milk directly with an equal volume of HCl in a porcelain casserole, giving the dish a slight rotary motion. In the presence of some dyes the separated curd acquires a pink coloration.

## CREAM

### 29 COLLECTION OF SAMPLE—OFFICIAL

Proceed as directed under **1.** The sample should be analyzed as soon as practicable and preferably not later than 3 days after taking.

### 30 PREPARATION OF SAMPLE—OFFICIAL

Immediately before withdrawing portions for the determinations, mix the sample by shaking, pouring, or stirring until it pours readily and a uniform emulsion has been secured. If the sample is very thick, warm it to 30–35°, and then mix. In case lumps of butter have separated, heat the sample to 38° or, if necessary, to 50° by placing in a warm water bath. Thoroly mix the portions for analysis and weigh immediately. (In commercial testing for fat by the Babcock method, it may be advisable to warm all samples to 38–50° in a water bath previous to mixing.) Avoid overheating the sample, thereby causing the cream to "oil off." This precaution is especially necessary in the case of a thin cream.

### 31 TOTAL SOLIDS—OFFICIAL

Proceed as directed under **5**, using 2–3 g of the sample.

### 32 ADDED WATER IN CREAM[11]—OFFICIAL, FIRST ACTION

Proceed as directed under **23**, but use the following formula to calculate the percentage of added $H_2O$:

$$W = \frac{\%\ \text{Serum in Cream}\ (T - T')}{T}, \text{ in which}$$

$W$ = the percentage of added $H_2O$;
$T$ = the freezing point of undiluted cream ($-0.550°$);
$T'$ = the observed freezing point of the given sample; and
$\%\ Serum = 100\% - (\%\ \text{fat} + \%\ \text{protein})$.

If protein is not determined it may be assumed to be 38% of the solids-not-fat.

### 33 ASH—OFFICIAL

Proceed as directed under **6**.

### 34 TOTAL NITROGEN—OFFICIAL

Proceed as directed under **7**.

## LACTOSE

### 35 *Gravimetric Method—Official*

Proceed as directed under **15**.

## FAT

### 36 *Roese-Gottlieb Method—Official*

Transfer 5 g of the sample to a Röhrig tube or a similar apparatus, dilute with $H_2O$ to about 10.5 cc, and proceed as directed under **16**.

### *Babcock Method[4]—Official*

### 37 REAGENTS

(**a**) *Sulfuric acid.*—Sp. gr. 1.82–1.83 at 20°.

(**b**) *Glymol, or clear white mineral oil.*—Sp. gr. not to exceed 0.85 at 20°. Oil-soluble artificial color may be added to the oil.

### 38 APPARATUS

(**a**) *Test bottles.*—The standard Babcock test bottles for cream shall be as follows:

(1) 50%, 6-*g*, *short-necked*, 6-*inch cream-test bottle.*—Total height 150–165 mm (5.9–6.5 inches). The bottom of the bottle shall be flat, and the axis of the neck shall be vertical when the bottle stands on a level surface. The charge of cream for the bottle shall be 9 g.

*Bulb.*—The capacity of the bulb to the junction with the neck shall be not less than 45 cc. The shape of the bulb shall be either cylindrical or conical. If cylindrical, the outside diameter shall be between 34 and 36 mm; if conical, the outside diameter of the base shall be between 31 and 33 mm, and the maximum diameter between 35 and 37 mm.

*Neck.*—The neck shall be cylindrical and of uniform diameter from at least 5 mm below the lowest graduation mark to at least 5 mm above the highest. The top of the neck shall be flared to a diameter of not less than 15 mm. The graduated portion of the neck shall have a length of not less than 63.5 mm. The total per cent graduation shall be 50. The graduations shall represent 5%, 1%, and ½%, respectively, from 0.0 to 50%. The 5% graduations shall extend at least half-way around the neck to the right; the ½% graduations shall be not less than 3 mm in length; and the 1% graduations shall be intermediate in length between the 5% and ½% graduations and shall project 2 mm to the left of the ½% graduations. Each 5% graduation shall be numbered (thus: 0, 5, 10, . . . 45, 50), the number being placed to the left of the scale. The capacity of the neck for each whole per cent on the scale shall be 0.1 cc. The maximum error in the total graduation or any part thereof shall not exceed the volume of the smallest unit of the graduation.

(2) *50%, 9-g, long-necked, 9-inch cream-test bottle.*—The same specifications shall apply to this bottle as to the 50%, 9-g, 6-inch cream-test bottle, except that the total height of this bottle shall be 210–229 mm (8.25–9.0 inches) and the graduated portion of the neck shall have a length of not less than 120 mm.

(3) *50%, 18-g, long-necked, 9-inch cream-test bottle.*—The same specifications shall apply to this bottle as to the 50%, 9-g, 9-inch cream-test bottle, except that the charge of cream for this bottle shall be 18 g.

Each bottle shall bear on the top of the neck above the graduations, in plain legible characters, a mark denoting the weight of the charge to be used, *viz.*, "9 g" or "18 g," as the case may be.

Each bottle shall be so constructed as to withstand the stress to which it will be subjected in the centrifuge.

(4) *Testing.*—Proceed as directed under **18(a$_1$)**.

**(b)** *Water bath for cream samples.*—Provided with a thermometer and a device for maintaining a temp. of 38–50°.

**(c)** *Cream weighing scales.*—With a sensibility reciprocal of 30 mg, *i.e.*, the addition of 30 mg to either pan of the scale, when loaded to capacity, shall cause a deflection of at least 1 subdivision of the graduation. The scales shall be set level upon a table support and be protected from drafts.

**(d)** *Weights.*—9 g and 18 g, respectively, and plainly marked "9 g" or "18 g," as the case may be. They shall be made of material capable of resisting corrosion or other injury, shall preferably be of a low squat shape, with rounded edges, and shall be verified at frequent intervals by comparison with standardized weights.

**(e)** *Acid measure.*—Described under **18(c)**.

**(f)** *Centrifuge or "tester."*—Described under **18(d)**.

**(g)** *Dividers or calipers.*—Described under **18(e)**.

**(h)** *Water bath for test bottles.*—Described under **18(f)**.

### 39 DETERMINATION

Weigh 9 g of the sample, prepared as directed under **30**, directly into a 9-g cream-test bottle, or 18 g into an 18-g bottle, and proceed by one of the following methods:

*Method 1*—After the cream has been weighed into the test bottle, add 8–12 cc of the $H_2SO_4$, in the case of the 9-g bottle, or 14–17 cc of the acid, in the case of the 18-g bottle, or add acid until the mixture of cream and acid, after shaking, has assumed a chocolate-brown color. Shake until all lumps have completely disappeared; then add 5–10 cc of soft $H_2O$ at 60° or above. Transfer the bottle to the centrifuge; counterbalance it; and, after the proper speed has been attained, whirl 5 min. Add hot $H_2O$ until the liquid column approaches the top graduation of the scale; then whirl 1 min. longer at a temp. of 55–60°. Adjust the temp., as directed under **19**, and, with the aid of dividers or calipers, measure the fat column, in terms of percentage by weight, from its lower surface to the bottom of the upper meniscus.

*Method 2.—For a 9-g bottle only.*—After the cream has been weighed into the test bottle, add 9 cc of soft $H_2O$ and thoroly mix; add 17.5 cc of the $H_2SO_4$ and shake until all lumps have completely disappeared. Transfer the bottle to the centrifuge; counterbalance it; and, after the proper speed has been attained, whirl 5 min. Fill the bottle to the neck with hot $H_2O$ and whirl 2 min. Add hot $H_2O$ until the liquid column approaches the top graduation of the scale; then whirl 1 min. longer at a temp. of 55–60°. Adjust the temp. and measure the fat column as directed under *Method 1.*

Whichever method is followed, the fat column, at the time of reading, should be translucent, of a golden yellow to amber color, and free from visible suspended particles. All tests in which the fat column is milky or shows the presence of curd or of charred matter, or in which the reading is indistinct or uncertain, should be rejected.

If glymol is used, a few drops only should be introduced into the bottle just before the reading is made; it must not be dropped in, but must be allowed to flow down the side of the neck. For the purpose of measurement, the surface separating the glymol and the fat is regarded as representing the upper limit of the column.

### 40 GELATIN—OFFICIAL

Proceed as directed under **26.**

### 41 PRESERVATIVES—OFFICIAL

Proceed as directed under **27** and under **XXXII.**

### 42 COLORING MATTERS—OFFICIAL

Proceed as directed under **28** and under **XXI.**

## EVAPORATED MILK (UNSWEETENED)

### 43 PREPARATION OF SAMPLE—OFFICIAL

**(a)** Transfer the entire contents of a can to a large dish, stir thoroly, and pass thru a fine sieve or strainer until a homogeneous mass is secured. If a slight separation of fat is evident, warm a portion of the sample containing the separated fat to 30–35° and agitate until a uniform emulsion is obtained; then combine with the unheated portion and mix thoroly. (If an appreciable quantitiy of fat has separated, rendering impossible the formation of a satisfactory emulsion, an accurate analysis cannot be made.)

**(b)** Dilute 40 g of the homogeneous mass prepared as directed under **(a)** with 60 g of $H_2O$ and mix thoroly.

### 44 TOTAL SOLIDS—OFFICIAL

Weigh 4–5 g of the diluted sample, **43(b)**, into a weighed flat-bottomed Pt dish, not less than 5 cm. in diameter, and proceed as directed under **5.** Correct the result for the dilution.

### 45 ASH—OFFICIAL

Ignite the residue from the total solids determination, **44,** at a low red heat until the ash is free from C. Correct the result for the dilution.

### 46 FAT—OFFICIAL

Weigh 4–5 g of the undiluted sample, **43(a)**, into a Röhrig tube or a similar apparatus; dilute with $H_2O$ to about 10.5 cc; and proceed as directed under **16.**

### 47 TOTAL NITROGEN—OFFICIAL

Weigh 5 g of the undiluted sample, **43(a)**; transfer to a Kjeldahl flask; and proceed as directed under **II, 19, 22** or **24.** Multiply the percentage of N by 6.38 to obtain the equivalent percentage of N compounds.

### 48 CASEIN—OFFICIAL

Weigh 10 g of the diluted sample, **43(b)**, into a beaker, and proceed as directed under **8** or **9**. Correct the result for the dilution.

### 49 ALBUMIN—OFFICIAL

Proceed as directed under **10**, using the filtrate from **48**. Correct the result for the dilution.

### 50 LACTOSE—OFFICIAL

Proceed as directed under **13** or **15**, using the diluted sample, **43(b)**, and correct the result for the dilution.

### 51 GELATIN—OFFICIAL

Proceed as directed under **26**.

### 52 PRESERVATIVES—OFFICIAL

Proceed as directed under **27** and under **XXXII**.

### 53 COLORING MATTERS—OFFICIAL

Proceed as directed under **28** and under **XXI**.

## SWEETENED CONDENSED MILK

### 54 PREPARATION OF SAMPLE—OFFICIAL

**(a)** If the can is cold, place it in $H_2O$ at 30–35° until warm. Open, scrape out all milk adhering to the interior of the can, and after transferring to a dish sufficiently large to permit stirring thoroly mix until the whole mass is homogeneous.

**(b)** Weigh 100 g of the thoroly mixed sample into a 500 cc volumetric flask, dilute to the mark with $H_2O$, and mix thoroly. If the sample will not emulsify uniformly, weigh out a separate portion of **(a)** for each determination.

### 55 TOTAL SOLIDS—OFFICIAL

Use 10 cc of the soln, prepared as directed under **54(b)**, and proceed as directed under **5**, drying on either sand or asbestos fiber. Correct the result for the dilution.

### 56 ASH—OFFICIAL

Evaporate 10 cc of the soln, prepared as directed under **54(b)**, to dryness on a water bath and ignite the residue as directed under **XXVII, 8**. Correct the result for the dilution.

### 57 PROTEIN—OFFICIAL

Determine the N as directed under **II, 19, 22,** or **24,** using 10 cc of the soln prepared as directed under **54(b)**, and multiply by 6.38 to obtain the equivalent of protein. Correct the result for the dilution.

### 58 LACTOSE—OFFICIAL

Dilute 100 cc of the soln, prepared as directed under **54(b)**, in a 250 cc volumetric flask to about 200 cc; add 6 cc of Fehling's $CuSO_4$ soln (**XXXIV, 31(a)**), make up to the mark; and mix thoroly. Filter thru a dry filter and determine lactose as directed under **XXXIV, 54** or **56**. Correct the result for the dilution.

### 59 FAT—OFFICIAL

Weigh accurately 4–5 g of the homogeneous sample, prepared as directed under **54(a)**, into a Röhrig tube or a similar apparatus; dilute with $H_2O$ to about 10.5 cc; and proceed as directed under **16**.

## SUCROSE[12]—OFFICIAL

### 60 REAGENT

To 220 g of yellow HgO, add 300–400 cc of $H_2O$ and sufficient $HNO_3$ to form a clear soln (about 140 cc), being careful to use the least possible excess of acid. Dilute to 800–900 cc and add 10% NaOH soln slowly and with constant shaking until a slight permanent precipitate is obtained. Dilute to 1 liter and filter. The soln tends to become acid with age owing to the deposition of basic mercuric salts. For this reason dilute alkali should be added occasionally until a slight permanent precipitate is formed and the soln filtered.

### 61 DETERMINATION

Introduce 50 cc of the soln, prepared as directed under **54(b)**, into a 100 cc volumetric flask; add 25 cc of $H_2O$; mix; add 5 cc of the $Hg(NO_3)_2$ reagent; and shake thoroly. Without delay and while shaking constantly, add sufficient 0.5 $N$ NaOH soln to render the mixture neutral to litmus paper, being careful to avoid an alkaline reaction (usually 12–13 cc will be required). Dilute to 100 cc with $H_2O$, mix thoroly, and filter thru a dry paper. Polarize the filtrate in a 200 mm tube, then invert at room temp. as directed under **XXXIV, 23(c)**, and polarize the inverted soln. Correct both readings for the volume occupied by the protein, **57**, and the fat, **59**, 1 g of protein occupying a space of 0.8 cc and 1 g of fat, 1.075 cc. Calculate the percentage of sucrose by the following formula, using the corrected direct and invert readings obtained above:

$$S = \frac{100\ (a-b)}{142.35 - \frac{t}{2}} \times \frac{26}{W}, \text{ in which}$$

$S$ = percentage of sucrose in the sample;
$a$ = corrected direct polarization;
$b$ = corrected invert polarization;
$t$ = temp. of soln polarized; and
$W$ = weight of sample taken (10 g).

## DRIED MILK AND MALTED MILK

### 62 PREPARATION OF SAMPLE—TENTATIVE

Sift the sample thru a 20-mesh sieve onto a large sheet of paper, rubbing the material thru the sieve and tapping vigorously if necessary. Grind the residue in a mortar, pass thru the sieve, and mix into the sifted material. Discard particles of wood and other material which cannot be ground. Sift the sample 2 more times, mixing thoroly each time. To avoid absorption of moisture, operate as rapidly as possible, and preserve the sample in an air-tight container.

## MOISTURE[13]—TENTATIVE

### 63 APPARATUS

*Metal dish.*—Diameter about 55 mm, height about 15 mm, provided with a slip-in inverted cover fitting tightly on the inside.

### 64 DETERMINATION

Weigh 1–1.5 g of the sample into the previously weighed metal dish, cover tightly, and reweigh. Dry in the loosely covered dish, placed in direct contact with the metal shelf of the vacuum oven, to constant weight (approximately 5 hours) under a pressure not to exceed 100 mm (4 inches) of Hg, at the temp. of boiling $H_2O$. During the drying admit into the oven a slow current of air (about 2 bubbles per second), dried by passing thru $H_2SO_4$. Discontinue the action of the vacuum pump and carefully admit dried air into the oven. Press the cover tightly into the dish, remove the dish from the oven, cool, and weigh. Calculate the percentage loss in weight as moisture.

**65**

### PROTEIN—TENTATIVE

Weigh 1 g of the sample into a Kjeldahl digestion flask and determine N as directed under **II, 24.** Multiply the N by 6.38 to obtain the equivalent of N compounds.

**66**

### ASH—TENTATIVE

Ignite 1 g of the sample at a low red heat until free from C. Cool in a desiccator and weigh.

**67**

### FAT (FOR MALTED MILK)[13]—TENTATIVE

Weigh accurately about 1 g of the well-mixed sample into a small, lipped beaker. Add 1 cc of $H_2O$ and mix well with a glass rod to form a thick liquid free from lumps. Add 10 cc more of $H_2O$, warm on the steam bath, and transfer to a Röhrig tube or similar apparatus. Cool, add 10 cc of 95% (by volume) alcohol, and mix. Add 25 cc of ethyl ether and proceed with the extraction as in the official Roese-Gottlieb method for milk, **16.** Dissolve the dried fat in petroleum ether and determine the quantity of any insoluble residue that may be present.

**68**

### FAT (FOR DRIED MILK)[15]—TENTATIVE

Proceed as under **67.** Add after the first extraction with ether 4 cc of 95% alcohol to the liquid remaining in the extraction apparatus, mix, and proceed with the second extraction. If necessary, in the third extraction add sufficient $H_2O$ to raise the level of the aqueous layer to its original volume. With whole milk and cream powders make a third extraction, using 15 cc of each ether.

**69**

### ROESE-GOTTLIEB METHOD (FOR DRIED MILK)[16]—TENTATIVE

Weigh out about 1 g of well-mixed sample into a small, lipped beaker. Add about 1 cc of $H_2O$ and mix well with a glass rod to form a thick liquid free from lumps. Add 9 cc more of $H_2O$ and 1 cc of $NH_4OH$, warm on the steam bath, and transfer to a Röhrig tube or similar apparatus. Cool, add 10 cc of 95% (by volume) alcohol, and mix. Add 25 cc of ethyl ether, shake vigorously for 30 seconds, and proceed as directed under **16.** For the second extraction add 4 cc of 95% alcohol, extract the liquid remaining in the tube, and again proceed as directed under **16.** Make the third extraction, if necessary, like the second, adding sufficient $H_2O$ to raise the aqueous layer to its original volume. Dissolve the dried fat in petroleum ether and determine the quantity of any insoluble residue that may be present. In case of whole milk and cream powders, make a third extraction, using 15 cc of each ether, and proceed as under **16.**

**70**

### MICROSCOPICAL IDENTIFICATION OF MALTED MILK AND ITS FLAVORED PRODUCTS[17]—TENTATIVE

Mount a small quantity of the material in a drop of mineral oil on a slide, apply the cover-glass, and examine the preparation at a magnification of approximately 200, using a microscope lamp with daylight glass as a source of light. Control the light intensity by the iris diaphragm because a too brilliantly lighted field hinders the recognition of details. (See pp. 232-235.)

## BUTTER

(The following methods are also applicable to renovated or process butter and margarine.)

### 72 DIRECTIONS FOR SAMPLING—OFFICIAL*

### 73 SAMPLING—OFFICIAL (FIRST ACTION)

(**a**) *Tub butter*—Insert an ordinary trough butter trier for practically its full length vertically thru the butter mass at a point approximately half-way between the center and the edge of the tub. Give the trier one complete turn and withdraw a full core. Transfer the core immediately to the sample container with the aid of a spatula. Do not include moisture adhering to the outside of the trier. When more than 1 core is taken from a tub, withdraw the cores at different points around the circumference of an imaginary circle half-way between the center and edge of the tub. Wipe the trier clean and dry after each core is removed. Fill the trier holes with a plug of butter approximately 1 inch long procured from an extra core. For butter stored at a moderate temp. use an unwarmed trier; for very hard butter use a warmed trier. Frozen butter should be stored 24 hours in a tempering room before sampling.

Sample as a minimum, in the case of—

(1) Tubs marked with churn batch numbers, 3 tubs from each batch in the lot. Take 1 core from each of 3 tubs in the batch. Composite the 3 full cores of each batch separately.

(2) Tubs not marked with churn numbers, the number of tubs indicated in the schedule below. Take for each sub-sample 3 cores from each tub.

(**b**) *Print butter.*—Withdraw as a minimum, sub-samples of at least ½ pound each, according to the schedule. Remove the wrapper and place each sub-sample in a separate sample container. If print butter is identified by batch number, sample as under tub (**a**), except to analyze the 3 sub-samples separately.

*Sampling Schedule*

| NO. OF UNITS (TUBS OR CASES) IN THE LOT | NO. OF UNITS TO BE SAMPLED |
|---|---|
| 1–10 | 3 |
| 11–25 | 5 |
| 26–50 | 6 |
| 51–75 | 8 |
| 76–100 | 10 |

Take a greater number of sub-samples from tub or print butter if circumstances indicate the necessity.

(**c**) *Sample containers.*—Use a glass jar, preferably with glass top, of such type as will prevent loss of moisture by evaporation or entrance of $H_2O$ into the jar. Tops containing a liner of any material should not be used.

### 74 PREPARATION OF SAMPLE—OFFICIAL

Soften the entire sample in a closed vessel at as low a temp. as possible. Shake vigorously until a perfectly homogeneous, semi-solid mass is obtained. Weigh the portions for analysis at once. If the sample is kept for any length of time, it must be softened and shaken until semi-solid before portions are withdrawn for analysis.

### 75 *Mechanical Stirrer Method*[18]*—Tentative*

Soften the sample, 250–500 g, in a closed vessel, to such an extent that on stirring for 2–3 min. the product will reach a temp. of 31–34°. Stir with a malted milk

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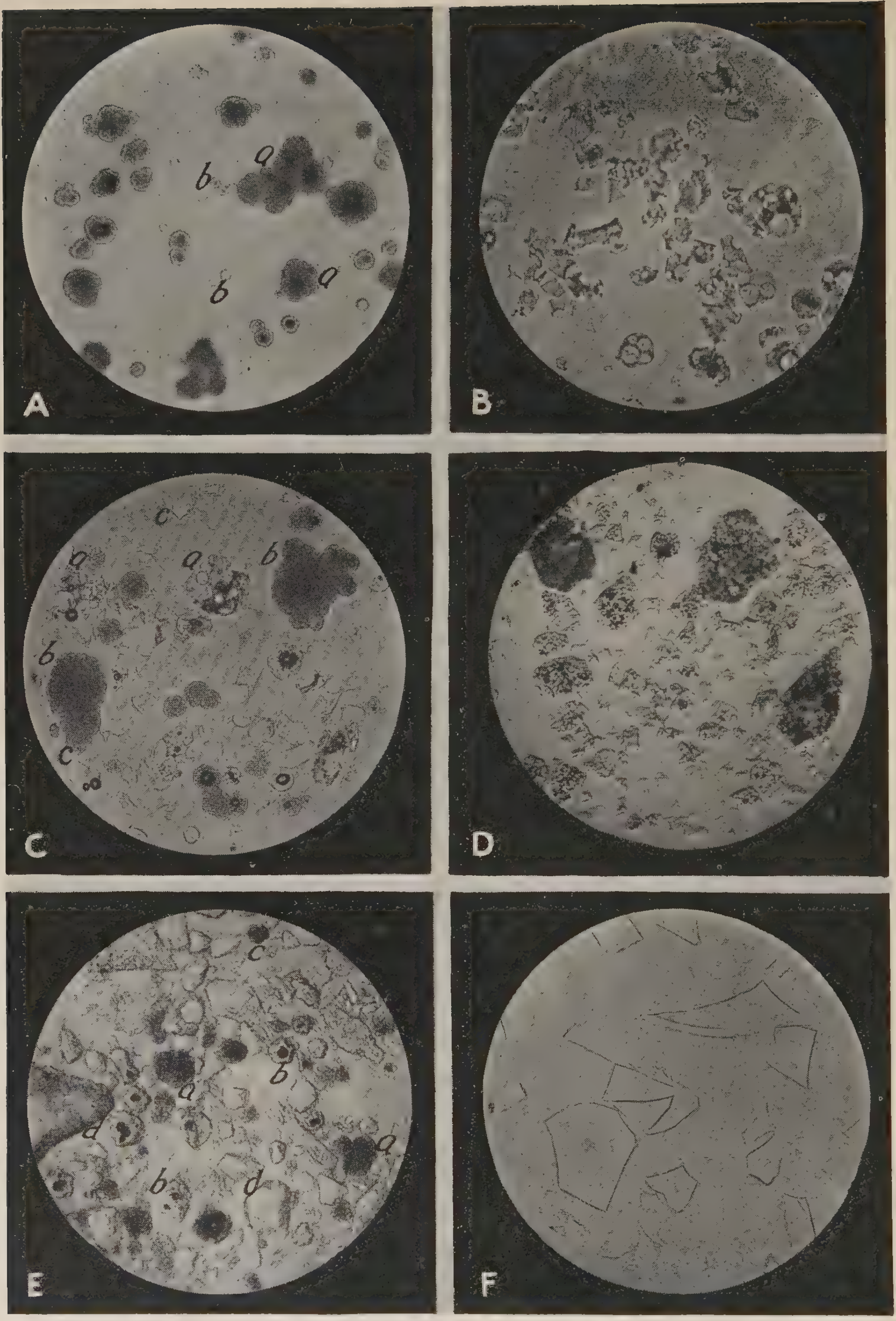
* See note 7, p. xvii.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F

FIG. 24.—PHOTOMICROGRAPHS IDENTIFYING MALTED MILK AND ITS ALLIED PRODUCTS

## DESCRIPTION OF THE PHOTOMICROGRAPHS

### FIGURE 24

A.—SPRAY-DRIED WHOLE MILK; (a) MILK MASSES, (b) FAT GLOBULES; B.—SPRAY-DRIED MALT EXTRACT; C.—MECHANICAL MIXTURE; (a) SPRAY-DRIED MALT EXTRACT, (b) MILK MASSES, (c) FAT GLOBULES; D.—SPRAY-DRIED SKIM MILK; E.—MECHANICAL MIXTURE; (a) SPRAY-DRIED SKIM MILK, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; F.—DRUM-DRIED MALT EXTRACT.

A represents a spray-dried whole milk. The large particles represent aggregates of globular milk masses having a stippled surface (a). The fat appears as droplets (b).

B represents a spray-dried malt extract having the appearance of aggregates of droplets enclosed in spherical masses.

C represents a product made by mixing the spray-dried whole milk and the spray-dried malt extract, shown in A and B, in the proportion necessary to give the approximate composition of malted milk. The globular stippled milk masses (b) and the malt extract masses (a) are easily recognized; (c) fat globules.

D represents a spray-dried skim milk that might be confused with the spray-dried malt extract (B) because the structure of the spherical masses is similar. A comparison of the 2 pictures, however, will show that the droplets in the malt extract masses are larger than those appearing in the milk masses.

E represents a product purchased on the market and represented to contain malt, skim milk, whole milk, cocoa, and sugar. Examination shows malt extract (b) dried skim milk (a), cocoa (c) and sugar (d) present. Whole milk is absent. The cocoa consists of brown amorphous particles, easily discernible under the microscope. The highly refractive, irregular fragments of sugar cannot be mistaken.

F represents a drum-dried malt extract. It consists of clear, highly refractive fragments closely resembling broken glass.

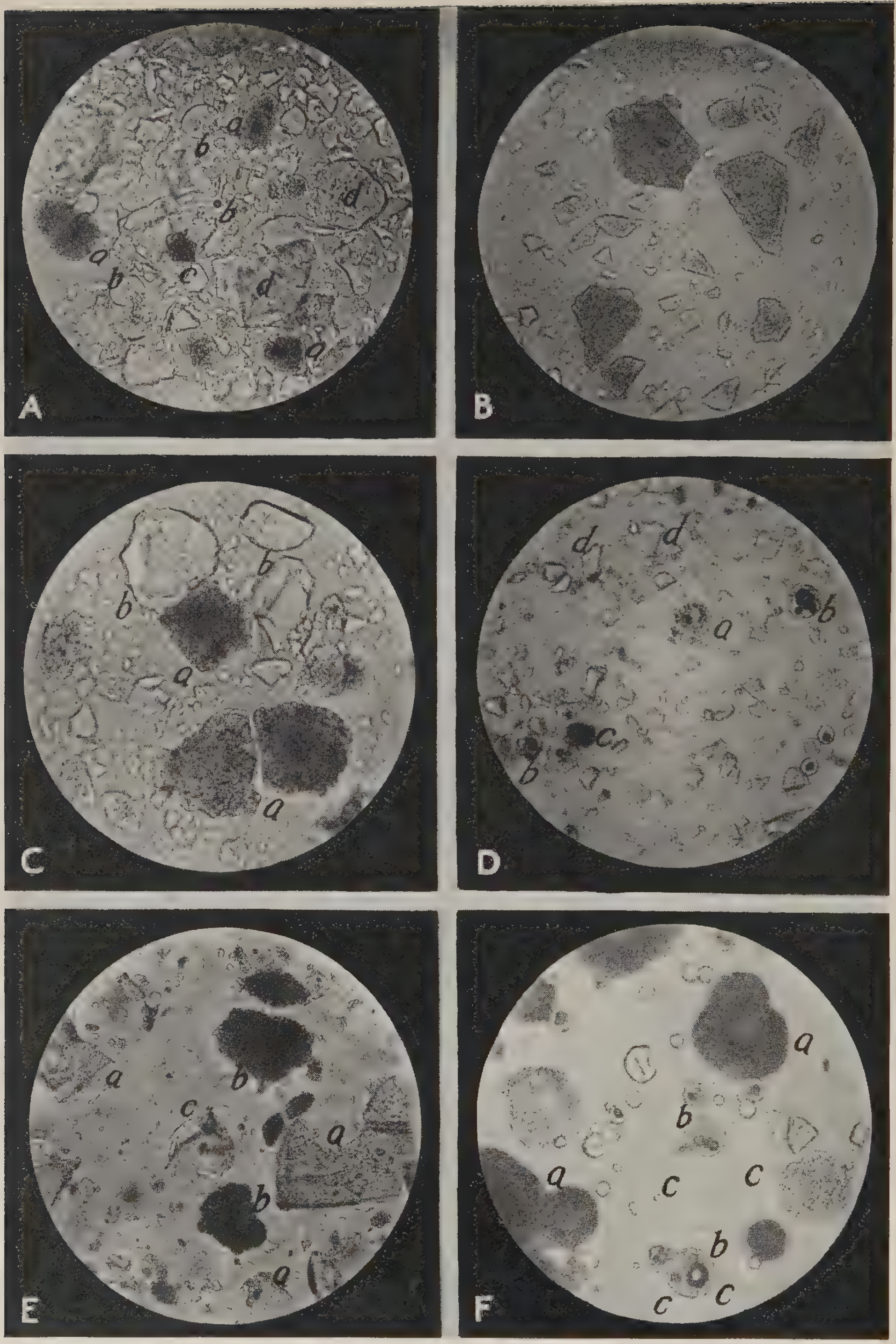
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FIG. 25.—PHOTOMICROGRAPHS IDENTIFYING MALTED MILK AND ITS ALLIED PRODUCTS

## DESCRIPTION OF THE PHOTOMICROGRAPHS

### FIGURE 25

A.—MECHANICAL MIXTURE; (a) MILK MASSES, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; B.—MALTED MILK; C.—SWEET CHOCOLATE FLAVOR MALTED MILK; (a) MALTED MILK MASSES, (b) SUGAR; D.—MECHANICAL MIXTURE; (a) SPRAY-DRIED SKIM MILK, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; E.—MECHANICAL MIXTURE; (a) MALTED MILK MASSES, (b) COCOA, (c) SUGAR; F.—PRODUCT PREPARED FROM MALT INFUSION AND MILK BY SPRAY DRYING; (a) MILK MASSES, (b) MALT EXTRACT MASSES, (c) FAT GLOBULES.

A represents a mechanical mixture of dried whole milk (A, Fig. 24), dried malt extract (B, Fig. 24), cocoa and sugar. The milk (a) and malt extract (b) masses and the sugar particles (d) are readily recognizable. A mass of cocoa appears near the center of the picture (c).

B is characteristic of the genuine malted milks of the market. This picture cannot be mistaken for any product of similar composition. The malt extract solids and the milk solids are incorporated into homogeneous irregular fragments having a stippled surface.

C represents a "sweet-chocolate-flavor malted milk" purchased on the market, prepared by simultaneously evaporating in vacuo milk, malt infusion, cocoa and sugar. It is easy to recognize the characteristic malted milk masses (a), shown in the picture immediately preceding. They are slightly thicker and appear darker in the picture because the cocoa is intimately associated with them.

D represents a mechanical mixture of spray-dried skim milk (D, Fig. 24), spray-dried malt extract (B, Fig. 24), cocoa and sugar. No trouble is experienced in identifying these materials.

E represents a mechanical mixture of malted milk (B), cocoa, and sugar. Examination shows that malted milk (a) is present.

F represents a product found on the market under the label "malted milk." It shows none of the characteristics found in genuine malted milk, as is readily seen by a comparison with B. Individual milk masses (a), fat globules (c), and malt extract masses (b) closely resembling the spray dried products (A and B,respectively, Fig. 24) predominate. Some particles show the stippled surface of genuine malted milk, but they are spherical instead of angular. A comparison of this picture with the picture showing the mechanical mixture of spray-dried whole milk and spray-dried malt extract (C, Fig. 24) shows a striking similarity.

stirrer for 2–3 min. with an up-and-down movement of the stirring device, at the same time slowly moving the vessel horizontally so that the stirrer reaches all parts of the sample. The final temp. must be 31–34°. If the temp. is below 31°, continue the softening and stirring until this temp. is reached. A temp. above 34° usually indicates that the sample has been softened too much, and is likely to separate. In this case, cool the sample until solid and repeat the softening and mixing. Weigh the portion for analysis within 3–4 hours if the room temp. is approximately 25°, or within ½ hour if the room temp. is 28° or above. Do not permit the butter to cool below 23° before weighing the portions for analysis.

### 76 MOISTURE—OFFICIAL

Weigh accurately 1.5–2.5 g of the sample into a flat-bottomed dish having a surface of at least 20 sq cm, dry at the temp. of boiling $H_2O$, and weigh at hourly intervals until the weight becomes constant. The use of clean, dry sand or asbestos is admissible.

### 77 MOISTURE, FAT, AND SALT[19]—TENTATIVE

#### APPARATUS

*Gooch crucible.*—Prepare a Gooch crucible of about 30 cc capacity with a 0.1 g pad of asbestos and place thereon 20 g of R. R. alundum, 90-mesh. (This is crystalline alumina especially prepared for C determinations. After use, again prepare the crucible for further use by igniting in a muffle, washing with $H_2O$, and drying at 100–105°.)

### 78 DETERMINATION

Weigh accurately in the weighed, specially prepared Gooch crucible 1.0–1.5 g of the prepared sample, dry for 2 hours at 100–105°, cool, weigh, and calculate the loss in weight as moisture. Extract the fat from the dried sample by placing the Gooch crubible in a closed-system extraction apparatus and extracting for 30–40 min. with $CCl_4$. Adjust the heat so that the solvent drops into the crucible at the same rate as the crucible drains and keep the crucible nearly full of the solvent. When the extraction is complete, remove most of the solvent remaining in the crucible by applying suction for a few seconds. Dry the crucible for 30 min. at 100–105°, cool, weigh, and calculate the percentage of non-fat solids. Calculate the percentage of fat by subracting the sum of the percentages of moisture and non-fat solids from 100.

To determine the salt, wash it out of the non-fat solids with $H_2O$ and titrate the aqueous soln with standard $AgNO_3$ soln, using $K_2CrO_4$ indicator.

### FAT

### 79 *I. Indirect Method—Official*

Dissolve the dry butter, obtained in the moisture determination in which no absorbent was used, in absolute ether or in petroleum ether; transfer to a weighed Gooch crucible with the aid of a wash bottle filled with the solvent; and wash until free from fat. Dry the crucible and its contents at the temp. of boiling $H_2O$ until the weight is constant and determine the fat by difference.

### 80 *II. Direct Method—Official*

From the dry butter, obtained in the determination of moisture either with or without the use of an absorbent, extract the fat with anhydrous, alcohol-free ether, or with petroleum ether (b.p. below 65°), receiving the soln in a weighed flask. Evaporate the ether, dry the extract at the temp. of boiling $H_2O$, and weigh at hourly intervals until the weight is constant

### 81 CASEIN, ASH, AND SALT—OFFICIAL

Cover the crucible containing the residue from the fat determination by the indirect method, **79**, and heat, gently at first; then raise the temp. gradually to just below redness. The cover may then be removed and heating continued until the contents of the crucible are white. The loss in weight represents casein, and the residue in the crucible, mineral matter. Dissolve this mineral matter in $H_2O$ slightly acidified with $HNO_3$ and determine Cl, either gravimetrically as directed under **XII, 33**, or volumetrically as directed under **XII, 35**, and calculate the NaCl.

### 82 SALT—OFFICIAL

Weigh in a counterpoised beaker 5–10 g of the sample; add about 20 cc of hot $H_2O$; and after the butter has melted transfer the whole to a separatory funnel. Insert the stopper and shake for a few moments. Let stand until all the fat has collected on the top of the $H_2O$; then draw off the $H_2O$ into a flask, being careful to let none of the fat globules pass. Again add hot $H_2O$, rinsing the beaker, and repeat the extraction 10 to 15 times, using 10–20 cc of $H_2O$ each time. The washings will contain all but a mere trace of the NaCl originally present in the butter. Determine the quantity in the whole or in an aliquot of the liquid by titration with standard $AgNO_3$ soln, using $K_2CrO_4$ indicator.

### 83 EXAMINATION OF FAT—OFFICIAL

Melt the butter and keep in a dry place at about 60° for 2–3 hours, or until the $H_2O$ and curd have entirely separated. Filter the clear, supernatant fat thru a dry filter paper in a hot water funnel or in an oven at about 60°. If the filtered liquid fat is not perfectly clear, refilter. Determine physical and chemical constants as directed under **XXXI.**

### 84 PRESERVATIVES—OFFICIAL

Proceed as directed under **27** and under **XXXII.**

### 85 COLORING MATTERS—OFFICIAL

Pour about 2 g of the filtered fat, dissolved in ether, into each of 2 test tubes. Into one of the tubes pour 1–2 cc of HCl (1+2), and into the other about the same volume of 10% NaOH soln. Shake the tubes well and allow to stand. In the presence of azo dyes the test tube to which the acid has been added will show a pink to wine-red coloration, while the alkaline soln in the other tube will show no color. If, on the other hand, annatto or other vegetable color is present, the alkaline soln will be colored yellow, while no color will be apparent in the acid soln.

*General test.*—Proceed as directed under **XXI**, particularly **3** and **21**, for the detection of oil-soluble coal tar dyes and annatto.

### 86 MICROSCOPIC EXAMINATION—OFFICIAL

**(a)** Place on a slide a small portion of the fresh, unmelted sample taken from the inside of the mass; add a drop of pure olive oil; apply a cover-glass with gentle pressure; and examine with a magnification of 120–150 diameters for crystals of lard, etc. Examine another portion of the sample with polarized light and selenite plate without the use of oil. Pure fresh butter will show neither crystals nor a parti-colored field with selenite. Renovated butter or other fats melted and cooled and mixed with butter will usually present crystals and variegated colors with the selenite plate.

**(b)** For further microscopic study dissolve in a test tube 3–4 cc of the fat in 15 cc of ether. Close the tube with a loose plug of cotton wool and allow to stand 12–24 hours at 20–25°. When crystals form at the bottom of the tube, remove with a pipet, glass rod, or tube; place on a slide; cover; and examine under a microscope. The crystals formed by later deposits may be examined in a similar way. Compare with crystals obtained in the same way from samples of known purity.

### 87 RENOVATED BUTTER[20] AND OLEOMARGARINE

### *I. Foam Test—Tentative*

Heat 2–3 g of the sample in either a spoon or a dish over a small flame. True butter will foam copiously, whereas process butter will bump and sputter like hot grease, with little or no foaming. Oleomargarine behaves like process butter, but chemical tests will determine whether the sample is oleomargarine or butter.

### 88 *II. Melted Fat Test—Tentative*

Melt 50–100 g of butter or renovated butter at 50°. The curd from butter will settle, leaving a clear supernatant fat; in the case of renovated butter, the supernatant fat remains more or less turbid.

## CHEESE

### 89 SELECTION AND PREPARATION OF SAMPLE—OFFICIAL

When the cheese can be cut, take a narrow, wedge-shaped segment reaching from the outer edge to the center of the cheese. Cut this into strips and pass 3 times thru a sausage machine. When the cheese cannot be cut, take the sample with a cheese trier. If only one plug can be obtained, take it perpendicularly to the surface of the cheese at a point ⅓ the distance from the edge to the center and extending either entirely or half way thru it. When possible draw 3 plugs, 1 from the center, 1 from a point near the outer edge, and 1 from a point half-way between the other two. For inspection purposes reject the rind, but for investigations requiring the absolute quantity of fat in the cheese include the rind in the sample. Grind the plugs in a sausage machine (the preferable method) or cut them very finely and mix thoroly.

### MOISTURE

### *Method I[21]—Official*

### 90 APPARATUS

*Metal dish.*—Diameter about 55 mm, height about 15 mm, provided with a slip-in inverted cover fitting tightly on the inside.

### 91 DETERMINATION

Weigh 2–3 g of the prepared sample into the previously weighed metal dish, cover tightly, and reweigh. In the case of soft cheese of high moisture content, weigh 1–2 g and partially dry on a steam bath. Dry the cheese in the loosely covered dish, placed in direct contact with the metal shelf of the oven, to constant weight (approximately 4 hours) under a pressure not to exceed 100 mm (4 inches) of Hg at the temp. of boiling $H_2O$. During the drying admit into the oven a slow current of air (about 2 bubbles per second) dried by passing thru concentrated $H_2SO_4$. Discontinue the action of the vacuum pump and carefully re-admit air into the oven. Press the cover tightly into the dish, remove the dish from the oven, cool, and weigh. Express the loss in weight as moisture.

### 92 *Method II[22]—Tentative*

Place in a flat-bottomed Pt dish, 6–7 cm in diameter, 10–15 g of washed and ignited sea sand (or 2–3 g of acid-washed and ignited short-fiber asbestos) and a glass rod about 5 mm in diameter and slightly longer than the diameter of the dish. Ignite, cool, and weigh the dish and its contents. Weigh into the dish 4–5 g of the prepared sample, and mix the cheese and sand or asbestos with the glass rod until the mass is practically homogeneous. (To facilitate the drying, leave the mass in as loose a condition as possible.) Dry to constant weight (3 hours is usually sufficient) in a water-jacketed oven at the temp. of boiling $H_2O$.

### 93 ASH AND SALT—OFFICIAL

The dry residue from the moisture determination may be used for the determination of ash. If the cheese is rich in fat, the asbestos will be saturated with it. Ignite cautiously to avoid spattering and remove the lamp while the fat is burning. When the flame has died out, complete the ignition in a muffle at low redness. When desired, the salt in the ash may be determined by dissolving the latter in $HNO_3$ (1+9), determining the Cl as directed under **XII, 33** or **35**, and calculating to NaCl.

### 94 NITROGEN—OFFICIAL

Determine N in an accurately weighed portion (about 2 g) of the prepared sample as directed under **II, 19, 22** or **24**. Multiply the percentage of N by 6.38 to obtain the percentage of N compounds.

### 95 ACIDITY—OFFICIAL

To 10 g of finely divided cheese add $H_2O$ at a temp. of 40° until the volume equals 105 cc, shake vigorously, and filter. Titrate 25 cc portions of the filtrate, as representing 2.5 g of the sample, with standard NaOH, preferably 0.1 *N*, using phenolphthalein indicator. Express the result in terms of lactic acid. 1 cc of 0.1 *N* NaOH soln = 0.0090 g of lactic acid.

### 96 COLORING MATTERS—TENTATIVE

Extract 25–50 g of the sample, prepared as directed under **89**, with ether; remove the ether by evaporation; and proceed as directed under **XXI, 3** or **19**.

### FAT

### 97 *Gravimetric Method—Official*

Cover the perforations in the bottom of an extraction tube with dry asbestos felt, on this place a mixture containing equal parts of anhydrous $CuSO_4$ and pure, dry sand to a depth of about 5 cm, packing loosely. Cover the upper surface of this material with a layer of asbestos, on which place a weighed portion (2–5 g, according to the fat content) of the sample and extract with anhydrous ether (**XXVII, 14**) for 5 hours in a continuous extraction apparatus. Remove the cheese to a mortar and grind it with pure sand to a fine powder, return the mixed cheese and sand to the extraction tube, wash the mortar with ether, add the washings to the tube, and continue the extraction for at least 10 hours. Evaporate the ether from the extract, dry at the temp. of boiling $H_2O$, cool in a desiccator, and weigh.

### 98 *Schmidt-Bondzynski Method, Modified—Official*

Rub up, by means of a glass rod, 1 g of the prepared sample with 9 cc of $H_2O$ and 1 cc of $NH_4OH$ in a narrow 100–125 cc beaker. Digest the mixture at a low heat until the casein is well softened; neutralize with HCl, using litmus as an indicator; and add 10 cc more of HCl. Add about 0.5 g of sand previously digested with HCl to prevent bumping and boil gently for 5 min., keeping the beaker covered with a watch-glass. Cool the soln, transfer to a Röhrig tube or a similar apparatus, rinse the beaker with 25 cc of ether, and transfer the ether rinsings to the Röhrig tube, shaking thoroly. Add 25 cc of petroleum ether (b. p. below 65°), shake thoroly, and let the mixture separate. Proceed from this point as directed under **16**, beginning with "Draw off as much as possible of the ether-fat soln."

### 99 EXAMINATION OF FAT—OFFICIAL

(**a**) *Alkaline extraction.*—Treat about 300 g of the cheese, cut into fragments the size of a pea, with 700 cc of 5% KOH soln at 20° in a large, wide-necked flask, shaking vigorously to dissolve the casein. In 5–10 min. the casein will be dissolved, and the fat will rise to the surface in lumps. Collect the lumps of fat into as large a mass as possible by shaking gently. Pour cold $H_2O$ into the flask until the fat is driven up into the neck and remove it by suitable means. Wash the fat thus obtained with just sufficient $H_2O$ to remove the residue of the alkali which it may contain. The fat is not perceptibly attacked by the alkali in this treatment, is practically all separated in a short time, and is then easily prepared for chemical analysis by filtering and drying as directed under **83.** Examine the fat as directed under **XXXI.**

(**b**) *Acid extraction.*—Pass the cheese thru a grinding machine; transfer to a large flask; and cover with warm $H_2O$, using 1 cc for every gram of cheese. Shake thoroly and add $H_2SO_4$ slowly and in small quantities, shaking after each addition of acid. The total quantity of acid used should be the same as the quantity of $H_2O$ employed. Remove the fat, which separates after standing a few min., by means of a separatory funnel; wash free from acid; filter; and dry as directed under **83.** Examine the fat as directed under **XXXI.**

## TARTARIC ACID[23]

### 100 *Qualitative Test—Tentative*

To 5 g of the ground cheese, add 40 cc of $H_2O$ at a temp. of about 50° and shake until the cheese is thoroly broken up. Add 3 cc of a 1% $H_2SO_4$ soln and shake vigorously; then add 2 cc of a 20% soln of phosphotungstic acid and again shake vigorously. Let stand for 5 min. and filter. To 25 cc of the filtrate add sufficient saturated $Ba(OH)_2$ soln to make alkaline and 25 cc of 95% (by volume) alcohol, shake vigorously, and allow to settle. Filter thru a Büchner funnel, using light suction, and wash the residue on the filter several times with $H_2O$. Transfer a portion of the paste to a small evaporating dish and dry on the steam bath. Add a few cc of concentrated $H_2SO_4$ and a few crystals of resorcin, and heat slowly. If tartaric acid is present, there is produced a rose-red color that is slowly discharged on dilution with $H_2O$.

### *Quantitative Method—Tentative*

### 101 REAGENTS

(**a**) *Hydrochloric acid.*—Approximately 2% soln. Dilute 47 cc of HCl to 1 liter with $H_2O$.

(**b**) *Sodium oxalate soln.*—Dissolve 2 g of Na-oxalate in 100 cc of $H_2O$.

(**c**) *Potassium chloride wash soln.*—Dissolve 15 g of KCl in 100 cc of $H_2O$ and add 20 cc of 95% (by volume) alcohol.

(**d**) *Tartaric acid soln.*—Dissolve 1.5 g of pure tartaric acid in previously boiled and cooled $H_2O$ and dilute to 100 cc at 20°. Titrate with 0.1 *N* NaOH soln to determine the quantity of tartaric acid in 10 cc of the soln.

(**e**) *Potassium chloride.*—Finely powdered.

### 102 DETERMINATION

Weigh 25 g of the ground cheese into a 500 cc wide-mouthed salt bottle and add, 25 cc at a time, 100 cc of $H_2O$ at a temp. of 50–60°, shaking vigorously after each addition. If necessary, continue the shaking until the cheese is thoroly broken up. Then add 25 cc of the Na-oxalate soln and shake vigorously for 1 min. Add 100 cc of the HCl soln, 25 cc at a time, shaking vigorously after each addition. Add 50 g of the powdered KCl and shake for 5 min. To avoid churning, keep the mixture warm (at about 50°) during the shaking. Transfer the contents of the bottle, with the aid of $H_2O$, to a 300 cc volumetric flask, cool to 20°, and make up to the mark with $H_2O$. Mix thoroly; let stand for 10 min., with occasional shaking; and then filter thru a dry folded filter, discarding the first few cc of the filtrate. Disregard any opalescence and transfer 200 cc of the filtrate to a 250 cc volumetric flask. Neutralize with 1 $N$ NaOH soln, using phenolphthalein indicator, and then add 5.2 cc in excess. Make up to the mark with $H_2O$, mix thoroly, let stand for a few min., and filter thru a dry folded filter, discarding the first few cc of the filtrate. To 100 cc of the filtrate in a 250 cc beaker add, with constant stirring, 10 cc of the tartaric acid soln, 2 cc of glacial acetic acid, and 23 cc of 95% (by volume) alcohol. Cool in an ice bath, stir vigorously until the cream of tartar begins to crystallize, and let stand in a refrigerator overnight. Prepare a Gooch crucible, having a removable disk, with a pad of asbestos about 10 mm thick. Decant most of the liquid thru this filter, wash the precipitate into the crucible with the KCl wash soln, and wash the beaker and precipitate 3 times, using 20–30 cc of the wash soln in all. Place the asbestos and precipitate in the beaker in which the precipitation was made and wash the crucible with about 50 cc of hot $H_2O$. Heat the soln to boiling and titrate the hot soln with 0.1 $N$ NaOH soln, using phenolphthalein indicator. Calculate the percentage of tartaric acid in the cheese by means of the formula—

$X = 14.26\ [0.015\ (B+1.5) - A]$, in which
$A$ = g of tartaric acid in 10 cc of the tartaric acid soln reagent; and
$B$ = cc of 0.1 $N$ NaOH soln required for the titration.

In the factor 14.26 the concentration caused by the insoluble solids of cheese of average composition is also taken into consideration.

### CITRIC ACID[24]

**103** *Qualitative Test—Tentative*

To 10 g of the ground cheese, add 20 cc of $H_2O$ at a temp. of about 50° and shake vigorously until the cheese is thoroly broken up. Add 20 cc of $H_2SO_4$ (1+1) and 2 cc of a 20% soln of phosphotungstic acid, and shake vigorously. Let stand for 5 min. and filter. To 20 cc of the filtrate add 10 cc of Br water and 5 cc of KBr soln and proceed with the oxidation as directed in the quantitative determination. Add sufficient $FeSO_4$ soln to dissolve the precipitated $MnO_2$. If citric acid is present, a heavy white precipitate that settles rapidly is formed.

### *Quantitative Method—Tentative*

**104** REAGENTS

(a) *Sulfuric acid.*—Approximately 1% soln. Dilute 6 cc of $H_2SO_4$ to 1 liter with $H_2O$.

(b) *Sodium oxalate soln.*—Dissolve 2 g of Na-oxalate in 100 cc of $H_2O$.

(c) *Finely powdered anhydrous sodium sulfate.*

(d) *Phosphotungstic acid soln.*—Dissolve 20 g of phosphotungstic acid in $H_2O$ and dilute to 100 cc.

(e) *Potassium bromide soln.*—Dissolve 15 g of KBr in 40 cc of $H_2O$.

(f) *Potassium permanganate soln.*—Dissolve 5 g of $KMnO_4$ in $H_2O$ and dilute to 100 cc.

(g) *Ferrous sulfate soln.*—Dissolve 20 g of $FeSO_4.7H_2O$ in 100 cc of $H_2O$ containing 1 cc of $H_2SO_4$.

(h) *Bromine water.*—A freshly prepared saturated soln.

### 105 DETERMINATION

Weigh 25 g of the ground cheese into a 500 cc wide-mouthed salt bottle, and add, 25 cc at a time, 100 cc of $H_2O$ at a temp. of 50–60°, shaking vigorously after each addition. If necessary, continue the shaking until the cheese is thoroly broken up. Then add 25 cc of the Na-oxalate soln and shake vigorously for 1 min. Add 100 cc of the 1% $H_2SO_4$ soln, 25 cc at a time, shaking vigorously after each addition. Add 3 cc of the phosphotungstic acid soln and shake; then add 25 g of the powdered $Na_2SO_4$, and shake for 5 min. To avoid churning, keep the mixture warm (at about 50°) during the shaking. Transfer the contents of the bottle with the aid of $H_2O$ to a 300 cc volumetric flask, cool to 20°, and make up to the mark with $H_2O$. Mix thoroly, let stand for 10 min. with occasional shaking, and then filter thru a dry folded filter, discarding the first few cc of the filtrate. Heat 200 cc of the filtrate to boiling and, while still hot, add 20 cc of $H_2SO_4$ (1+1) and 2 cc of the phosphotungstic acid soln. Mix and allow to stand for 15 min. With the aid of $H_2O$ transfer the mixture to a 250 cc volumetric flask, cool to 20°, make up to the mark with $H_2O$, and filter thru a dry folded filter. Transfer 100 cc of the clear filtrate to a 500 cc Erlenmeyer flask containing about 0.3 g of washed and dried asbestos. Add 10 cc of the Br water and 5 cc of the KBr soln, mix thoroly, and heat to 48–50°. Hold at this temp. for 5 min., add 25 cc of the permanganate soln, shake, and allow to stand for about 5 min. Cool the flask and contents to about 8°, add 40 cc of the cold $FeSO_4$ soln, shake continuously for 5 min., and let the mixture stand overnight in the refrigerator. Decant the supernatant liquid thru a Gooch crucible, measure the volume of the filtrate (a) and wash the precipitate into the crucible with this filtrate. Wash the precipitate with 3 successive 20 cc portions of ice-cold $H_2SO_4$ (1+100), sucking dry after each addition, and finally wash with 3 successive 20 cc portions of ice-cold $H_2O$. Dry the precipitate to constant weight over $H_2SO_4$ in a vacuum desiccator, protecting the precipitate from strong light or, to save time, dry in a current of air passed thru $H_2SO_4$. Weigh, and remove the pentabromacetone by extracting first with 3 successive 20 cc portions of 95% (by volume) alcohol and then with 3 successive 20 cc portions of ether. Dry, and weigh the crucible. To the weight of the pentabromacetone add 0.004 g for each 100 cc of filtrate (a) to compensate for solubility of the pentabromacetone and multiply the result by 6.06 to obtain the percentage of anhydrous citric acid in the cheese. (In this factor consideration is taken of the concentration caused by the insoluble solids in 25 g of cheese. It is assumed that the solids of cheese are almost insoluble under the conditions maintained and that the average process cheese contains about 60% of solids. No allowance is made for variation in the salt or moisture content or for variation in the specific volume of the solids, as such variations do not appreciably affect the results.)

## ICE CREAM (PLAIN)

### 106 PREPARATION OF SAMPLE—OFFICIAL

Allow the sample to soften at room temp. Owing to the fact that melted butter fat tends to separate out and rise to the surface, it is not advisable to soften the ice cream by heating on a water bath or over a flame. Mix thoroly by stirring with a spoon or egg beater or by pouring back and forth between beakers.

### 107 *NITROGEN

Place 4–5 g of the sample in a digestion flask and proceed as directed under **II, 24.**

### FAT

### 108 *Roese-Gottlieb Method—Official*

Weigh 4 g of the thoroly mixed sample into a small dry beaker; add 3 cc of $H_2O$; thoroly mix with a glass rod; and transfer to a Röhrig tube or a similar apparatus, washing out the remaining portion with the aid of an additional 3 cc of $H_2O$. Add 2 cc of $NH_4OH$, mix thoroly, and heat in a water bath at 60°. From this point proceed as directed under **16**, beginning with "Add 10 cc of 95% alcohol and mix well."

### 109 COLORING MATTERS—TENTATIVE

Curdle 150–200 g of the melted sample by adding an equal volume of $H_2O$ and 10–20 cc of acetic acid. Heat the mixture to 70–80°, stirring meanwhile, and allow to cool. Continue as directed under **28** and **85** and under **XXI**, particularly **3**, and **21**, for the detection of oil-soluble coal tar dyes and annatto.

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[16] Ibid., **8**, 482 (1925); **13**, 42 (1930).
[17] Ibid., **12**, 238 (1929).
[18] Ibid., **11**, 276 (1928).
[19] Ibid., 39.
[20] U. S. Dept. Agr. Farmers Bull., **131**, p. 6; Bur. Chem. Circ. **19**, rev., p. 1 (1905).
[21] J. Assoc. Official Agr. Chem., **5**, 498 (1922).
[22] Ibid., **9**, 44 (1926).
[23] Ibid., **11**, 40 (1928).
[24] Ibid., **10**, 264 (1927); **11**, 41 (1928).

# XXIII. EGGS AND EGG PRODUCTS

## 1 COLLECTION AND PREPARATION OF SAMPLE[1]—TENTATIVE

No simple rules can be made for the collection of a sample representative of the average of any particular lot of egg material as the conditions encountered may differ widely. Experienced judgment must be used in each instance. If large lots are under examination, it is best to draw a number of samples for separate analyses rather than to attempt to get one composite representative sample.

(a) *Liquid eggs.*—Secure representative container or containers. Mix the contents of a container thoroly and draw about 300 g. A long-handled dipper or ladle serves well. Keep the sample hermetically sealed in a jar in a cool place. Report odor and appearance.

(b) *Frozen eggs.*—Secure representative container or containers. Examine contents as to odor and appearance. The condition of the contents can be determined best by boring a hole to the center of a container with an auger and noting the odor as the auger is withdrawn. If impossible to secure individual containers, samples may consist of the composite of the borings from the contents of each container. Take the borings midway between the center and circumference of the top of the can from at least 3 widely separated parts and extend them as near the bottom of the can as possible. Collect about 300 g of the sample. Keep the sample hermetically sealed in a jar in a cool place and in a frozen state if possible. Before analyzing warm the sample in a bath held below 50° and mix well.

(c) *Powdered dried eggs.*—Secure representative container or containers. For small packages, take entire parcel or parcels for the sample. For boxes and barrels, remove the top layer to a depth of about 6 inches with a flour scoop or other convenient instrument. Draw small quantities of sample totaling about 300–500 g from accessible parts of the container and place in a hermetically sealed jar. Report odor and appearance. Prepare the sample for analysis by mixing 3 times thru a domestic flour sifter to assure complete breaking up of lumps. Keep in a hermetically sealed jar in a cool place.

(d) *Flaked and drum dried eggs.*—Collect the sample as directed for powdered dried eggs. Report odor and appearance. Prepare albumin samples for analysis by grinding in a mill to pass entirely a 60-mesh sieve, and whole egg and yolk samples to pass entirely a 20-mesh sieve or as fine as is practicable. Keep in a hermetically sealed jar in a cool place.

## TOTAL SOLIDS

### *I. Vacuum Method[2]—Official*

### APPARATUS

2

(a) *Metal dish.*—Diameter about 55 mm, height about 15 mm, and provided with an inverted slip-in cover fitting tightly on the inside.

(b) *Air-tight desiccator.*—Should contain a fresh efficient desiccant.

(c) *Vacuum oven.*—Connected with a pump capable of maintaining a partial vacuum in the oven with a pressure equivalent to 25 mm or less of Hg and provided with a thermometer passing into the oven in such a way that the bulb is near the samples. Connect a concentrated $H_2SO_4$ gas drying bottle with the oven for admitting dry air to release the vacuum.

(d) *Mercury manometer.*—Used to indicate the pressure of the partial vacuum.

### 3 DETERMINATION

(**a**) *Dried eggs.*—Weigh accurately about 2 g of the well-mixed sample in a covered dish that previously has been dried at 98–100°, cooled in the desiccator, and weighed soon after attaining room temp. Loosen the cover (do not remove) and heat at 98–100° to constant weight (approximately 5 hours) in a partial vacuum having a pressure equivalent to 25 mm or less of Hg. Admit dry air into the oven to bring to atmospheric pressure. Immediately tighten the cover on the dish, transfer to desiccator, and weigh soon after room temp. is attained. Report the weight of egg residue as total solids.

(**b**) *Liquid or frozen eggs.*—Weigh accurately about 5 g of the sample in a covered dish that previously has been dried at 98–100°, cooled in the desiccator, and weighed soon after attaining room temp. Remove the cover and drive off most of the $H_2O$ by heating on the steam bath. Replace the cover loosely and complete the drying in a partial vacuum as directed under (**a**).

## ORGANIC AND AMMONIACAL NITROGEN[3]—OFFICIAL

### 4 PREPARATION OF SAMPLE

(**a**) *Powdered dried eggs.*—Transfer about 1 g of the well-mixed sample, accurately weighed, to a 500 cc Kjeldahl flask.

(**b**) *Liquid eggs.*—Weigh 2–3 g of the well-mixed sample by difference into a 500 cc Kjeldahl flask.

### 5 DETERMINATION

Determine N as directed under **II, 19,** or preferably **22** or **24.** (Complete digestion of the sample is accomplished most rapidly by the Kjeldahl-Gunning-Arnold method.) Distil the $NH_3$ into 30–50 cc of 0.1 *N* standard acid.

## WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40% ALCOHOL[4]—TENTATIVE

### 6 REAGENTS

(**a**) *Sodium chloride soln.*—Disssolve 28 g of NaCl in $H_2O$ and dilute to 300 cc.

(**b**) *Cresol red indicator.*—Grind 0.1 g of the dry powder in an agate mortar with 5.3 cc of 0.05 *N* NaOH soln. When soln is complete, dilute to 25 cc with $H_2O$. Dilute 5 cc of the stock soln to 50 cc with $H_2O$.

### 7 PREPARATION OF SOLUTION

(**a**) *Powdered eggs.*—Place approximately 2 g of sample into an 8-ounce nursing bottle. Add 25 cc of ethyl ether, stopper with a water-soaked cork, and mix thoroly but gently for several min. Centrifugalize until the supernatant liquid is clear and carefully decant off the ether soln, allowing none of the egg to be carried along. Re-extract 3 times with 20 cc portions of ether in the same manner. Dry the fat-free residue by aid of suction and reduce to a fine powder by working with a glass rod. Add 100 cc of $H_2O$ from a pipet, mix well to avoid lumping, and add exactly 100 cc more of $H_2O$. Mix the contents of the stoppered bottle gently on a slowly revolving wheel or by hand for 1 hour. The temp. of the $H_2O$ should not exceed 30°. Centrifugalize to facilitate filtration and filter thru a thin asbestos pad in a Hirsch funnel, using light suction. Proceed as directed under **8.**

(**b**) *Liquid eggs.*—Weigh accurately, by difference, approximately 10 g of the well-mixed sample into a 250 cc volumetric flask containing 150 cc of $H_2O$ and mix gently. For whites or for yolks, fill to the mark with $H_2O$, shake gently, and filter thru an 18½ cm fluted filter paper. If the filtrate is cloudy, allow to filter until drops of the filtrate become clear. Return the cloudy filtrate to the filter and wash the receiving container twice with clear filtrate, returning the washings to the filter. For whole eggs or for mixed whites and yolks, add 5 cc of 0.01 *N* acetic acid soln, mix gently, remove 1 drop of the egg soln to a spot plate containing 2 drops of $H_2O$, and add 1 drop of cresol red indicator. Repeat the addition of 5 cc of acetic acid soln, mixing and testing with the indicator on the spot plate until the coloration disappears. Fill to the mark with $H_2O$, shake gently, and filter thru an 18½ cm fluted filter paper, returning the filtrate to the filter if it is cloudy.

### 8 DETERMINATION

Determine N in 50 cc of the filtrate as directed under **II, 24,** making certain that the free flame does not touch the flask above the level of the liquid. Heat with a moderate flame for one hour after clearing. Distil into 20 cc of 0.1 *N* acid. Run a blank on the reagents.

Pipet off 100 cc of the above filtrate into a 200 cc flask, add 15 cc of the NaCl soln, and fill almost to the mark with ethyl alcohol. Mix, cool to room temp., make up to volume with alcohol, mix well, and allow to stand overnight. Pipet off the supernatant liquid and filter thru an 18½ cm fluted filter paper. Determine N in 100 cc of the filtrate as above. To avoid bumping, add glass beads or delay the addition of $K_2SO_4$ and HgO until the alcohol has been boiled off. Subtract the value obtained from the water-soluble N to obtain the water-soluble N precipitable by 40% alcohol.

## FAT (ACID HYDROLYSIS METHOD)[5]—TENTATIVE

### 9 PREPARATION OF SOLUTION

*Liquid eggs.*—Weigh accurately by difference approximately 5 g of the well-mixed sample into a 50 cc beaker, add 10 cc of HCl, and mix well. Set the beaker in a water bath held at 75–80°, and stir at frequent intervals for 15–25 min., or until the sample is sufficiently hydrolized to form a clear soln. Add 10 cc of 95% alcohol and cool.

*Powdered dried eggs.*—Weigh accurately 2 g of the well-mixed sample into a 50 cc beaker, add 2 cc of 95% alcohol, and stir to moisten all particles (the moistening of the sample with alcohol prevents lumping on addition of the acid). Add 10 cc of HCl (25+13), mix well, set the beaker in a water bath held at 75–80°, and stir at frequent intervals for 15–25 min., or until the sample is sufficiently hydrolized to form a clear soln. Add 10 cc of 95% alcohol and cool.

### 10 DETERMINATION

Transfer the mixture to a Röhrig or Mojonnier fat extraction apparatus. Rinse the beaker into the extraction tube with 25 cc of ethyl ether in 3 portions and shake the mixture well. Add 25 cc of redistilled petroleum ether (b. p. below 60°) and mix well. Let stand until the ether layer is practically clear. Thru a filter consisting of a pledget of cotton packed just firmly enough in the stem of a funnel to allow free passage of the ether, draw off as much as possible of the ether-fat soln into a weighed 125 cc beaker-flask containing some porcelain chips. Before weighing the beaker-flask, dry it in an oven at the temp. of boiling $H_2O$ and then allow it to stand in the air to constant weight. Re-extract the liquid remaining in the tube twice, each time with only 15 cc of each ether. Shake well on the addition of each ether. Draw off the clear ether solns thru the filter into the same flask as before and wash the tip of the spigot, the funnel, and the end of the funnel stem with a small quantity of a mixture of the two ethers in equal parts and free from suspended $H_2O$. Evaporate the ethers slowly on a steam bath, then dry the fat in a boiling-water oven to constant weight (approximately 90 min.). Remove the fat-flask from the oven, allow it to stand in the air until no further change in weight takes place, and weigh. Correct this weight by a blank determination on the reagents used.

## 11 LIPOIDS[6]—TENTATIVE

(a) *Liquid eggs.*—Weigh accurately by difference approximately 10 g of the well-mixed sample into a 200 cc nursing bottle, add 100 cc of anhydrous ether, stopper with a softened cork, and shake vigorously. Add five 5 cc portions of 95% alcohol and shake after each addition. (The gradual addition of alcohol with shaking coagulates the proteins to a very fine state.) Centrifugalize and decant the liquid into a 250 cc beaker containing some bits of broken porcelain. Wash the neck of the bottle with ether, and place the beaker with the fat soln on the steam bath. Add 15 cc of 70% alcohol to the egg residue in the bottle in a manner to wash down any particles adhering to the sides and set in a water bath held at 70–80° for 15 min. Shake occasionally with a rotary motion so as to moisten all particles with the alcohol. Cool, add 30 cc of ether, stopper, shake for 5 min., centrifugalize to throw down suspended particles, and decant the liquid into the original 250 cc beaker. Rinse the bottle neck with ether. Re-extract the residue with 2 successive 20 cc portions of ether, shaking 1 min. each time, centrifugalize, and decant into the beaker containing the first extract. Evaporate the combined ether-alcohol extracts just to dryness on the steam bath. Drive off any remaining apparent moisture on the sides of the beaker by placing in a boiling water oven for about 5 min. Dissolve the dried extract in about 15 cc of $CHCl_3$ and filter the soln into a previously dried and weighed flat-bottomed Pt dish thru a pledget of cotton packed in the stem of a funnel. Free any solid extract adhering to the beaker with a glass rod and transfer thru the filter into the Pt dish by means of $CHCl_3$ from a wash-bottle all soluble extract from the beaker bottom and sides. Finally wash the funnel and stem tip. (The filtrate should be perfectly clear.) Evaporate the $CHCl_3$ on the steam bath (an electric fan may be used to hasten evaporation) and dry the dish and contents in a boiling water oven to constant weight (approximately 90 min.). Weigh, and report the extract as lipoids.

(b) *Powdered dried egg.*—Transfer about 2 g of well-mixed sample, accurately weighed, to a funnel having a pledget of cotton loosely placed in the stem. Wash with ether 4 or 5 times to extract most of the ether-soluble substances. Collect the washings in a 250 cc beaker containing some bits of broken porcelain and place on the steam bath. Transfer the residue and cotton in the funnel to a small glass mortar and allow the ether to evaporate at room temp. Add 2–3 g of precipitated $CaCO_3$ of the egg residue, grind to a fine powder, and transfer the mixture to a 200 cc nursing bottle. Wash the mortar, pestle, funnel, and funnel-stem tip with ether and add the washings to the original ether extract. Continue as directed under (a), beginning with "Add 15 cc of 70% alcohol to the egg residue in the bottle."

## 12 LIPOID PHOSPHORIC ACID ($P_2O_5$)[7]—TENTATIVE

Dissolve the lipoids in 10–15 cc of $CHCl_3$, add 10–20 cc of 4% alcoholic KOH soln, evaporate to dryness on a steam bath, and char completely in a furnace at a faint red heat. Cover the dish with a cover-glass, add sufficient $HNO_3$ (1+3) to make the soln slightly acid, and filter into a 100 cc volumetric flask. Wash the filter and residue carefully, make up the filtrate to 100 cc, and determine the $P_2O_5$ as directed under **II, 7** or **10.** For the volumetric method pipet 20 cc of the soln into a 250 cc beaker, neutralize with $NH_4OH$ (1+3), and then slightly acidify with $HNO_3$ (1+3). Set the beaker in a water bath held at 45–50° and add 15 g of $NH_4NO_3$. When the soln has reached the temp. of the bath, add sufficient $NH_4$-molybdate soln, previously heated to 45–50°, to precipitate all the phosphates; stir; and heat for 30 min. Filter the precipitate on an asbestos mat in a Hirsch funnel, wash with cold $H_2O$, and proceed as directed under **II, 10** (a), beginning with "Transfer the precipitate and filter to the beaker or precipitating vessel." Report as lipoid $P_2O_5$.

### 13 UNSAPONIFIABLE MATTER[8]—TENTATIVE

Extract the lipoids according to the directions given in **12.** Determine the unsaponifiable matter in the extracted lipoids by the official F. A. C. method (**XXXI, 37**).[8]

## METHODS FOR DETECTION OF DECOMPOSITION

### ACIDITY OF FAT[9]—OFFICIAL

(Not applicable to egg white)

### 14 REAGENTS

(a) *Anhydrous ether.*—Prepare in the usual way from ordinary ethyl ether.

(b) *Benzene.*—Use the best available quality of benzene. If it is not neutral, titrate 50 cc with the 0.05 *N* Na-ethylate and correct subsequent results accordingly.

(c) 0.05 *N sodium ethylate.*—Dissolve a piece of metallic Na, approximately 1 cc in volume, in 800 cc of absolute alcohol. Titrate 10 cc of 0.1 *N* HCl with this soln and add the calculated volume of absolute alcohol to make the soln 0.05 *N*. Ascertain the normality factor by titration against 0.1 *N* HCl on the day the soln is used.

### 15 DETERMINATION

(a) *Dried eggs.*—Weigh in a tared Al dish about 63 mm (2½ inches) in diameter 2 g of the powdered sample and dry at 55° under a pressure not exceeding 125 mm (5 inches) of Hg. Weigh to the third decimal place at the end of 2 hours and make further weighings at ½-hour intervals until no further loss in weight occurs. Extract the dried residue with anhydrous ether, preferably in a Knorr apparatus. Carefully transfer the egg powder to a 12.5 cm hardened filter paper, fold the paper once, place it on a 15 cm qualitative filter paper, and roll the papers and contents into a cylinder that will fit snugly into the extraction tube, folding in one end of the cylinder to prevent loss of material. (An asbestos plug is not needed in the extraction tube, and if the extractor is working rapidly, 3 hours is sufficient to insure proper extraction.) Evaporate the ether from the extraction flask, dry the extract for 1 hour at 55° under a pressure not exceeding 125 mm, and weigh to the third decimal place. Dissolve the extract in 50 cc of benzene, add 3 to 4 drops of phenolphthalein indicator, and titrate with the standard Na-ethylate soln. The end point is reached when the yellow color changes to orange. Express the result as the number of cc of 0.05 *N* Na-ethylate required per g of ether extract.

(b) *Liquid eggs.*—Weigh to the third decimal place in a weighed lead dish about 5 g of the sample and dry as directed under (a). Weigh after drying for about 5 hours and thereafter, at 1 hour intervals, until no further loss in weight occurs. To prepare the dried residue for extraction with ether, place the lead dish upon a 12.5 cm hardened filter paper, cut the sides of the dish thru at 4 equidistant points, and flatten down. Place another similar filter paper on top of the lead dish and its contents and roll the papers and dish into a cylinder that will fit snugly into the extractor, folding in one end of the cylinder to prevent any of the egg residue from dropping into the extraction flask. Proceed thereafter as directed under dried eggs.

### PHOSPHORIC-PENTOXIDE[10]—TENTATIVE

### 16 REAGENTS

(a) *Sodium carbonate soln.*—Dissolve 10 g of $Na_2CO_3$ in $H_2O$ and dilute to 100 cc.

(b) *Olive oil.*

### 17 PREPARATION OF SOLUTION

From the well-mixed sample, weigh accurately, by difference, approximately 2 g of yolk, 4 g of whole eggs, or 10 g of whites into a 250 cc low-form Pyrex beaker. Add 20 cc of the $Na_2CO_3$ soln and evaporate to dryness on an electric hot plate or overnight at 100–105°. Add to the whites 0.5 cc of olive oil. Transfer the beaker while hot to an electric muffle heated to 500° (faint redness), and allow it to remain at this temp. for 1 hour. Cool, add a few drops of $H_2O$, break up the charge with a glass rod with a flattened end, and cover the beaker with a watch-glass. Then add slowly and with continuous stirring 10 cc of $HNO_3$ (1+3) and filter, collecting the filtrate in a 300 cc or 500 cc Erlenmeyer flask. Thoroly wash the charred material and filter with $H_2O$ from a wash-bottle.

### 18 DETERMINATION

In the prepared filtrate determine the $P_2O_5$ as directed under **12** and II, **10** (**a**), using 40–50 cc of the molybdate soln. Report as total $P_2O_5$.

## CHLORINE[10]—TENTATIVE

### 19 REAGENTS

(**a**) *Sodium carbonate soln.*—Dissolve 10 g of $Na_2CO_3$ in $H_2O$ and dilute to 100 cc.
(**b**) *Olive oil.*

### PREPARATION OF SOLUTION

*Liquid Eggs.*—From the well-mixed sample weigh accurately, by difference, approximately 4 g of yolk or 7 g of whole eggs or 10 g of whites into a 150 cc low-form Pyrex beaker, add 20 cc of the $Na_2CO_3$ soln, mix, and evaporate to dryness on an electric hot plate or overnight at 100°. Transfer the beaker while hot to an electric muffle heated to 500° (faint redness), and allow it to remain at that temp. for 1 hour. Cool, add a few drops of $H_2O$, and break up the charge with a glass rod. Add 50 cc of $H_2O$, cover the beaker with a watch-glass, add slowly 20 cc of $HNO_3$ (1+3), mix, remove the watch-glass, and filter, collecting the filtrate in a 200 cc volumetric flask. Wash the charred material and filter thoroly with $H_2O$ from a wash bottle, keeping the total volume of filtrate to 180 cc or less.

*Dried Eggs.*—From the well-mixed sample, transfer 2 g of whole eggs or yolks, or 1 g of whites into a 150 cc low-form Pyrex beaker, and proceed as directed under *Liquid Eggs.*

### DETERMINATION

Add 10 cc of 0.1 *N* silver nitrate soln to the prepared filtrate and proceed as directed under **XII, 35.**

### 22 EXTRACTION AND IDENTIFICATION OF ADDED COLOR

Determine as directed under **XX, 72.**

### 23 DETECTION OF THE PRESENCE OF WHOLE EGG OR COMMERCIAL EGG YOLK SOLIDS

Determine as directed under **XX, 73.**

### 24 ESTIMATION OF THE PERCENTAGE OF EGG SOLIDS

Determine as directed under **XX, 74.**

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[4] Ibid., **7,** 85 (1924); **8,** 620 (1925); **12,** 56 (1929); **14** (1931).
[5] Ibid., **8,** 601 (1925); **9,** 58 (1926).
[6] Ibid., **7,** 91 (1923); **8,** 602 (1925); **9,** 58 (1926).
[7] Ibid., **7,** 91 (1923); **8,** 603 (1925); **9,** 59 (1926).
[8] Ibid., **9,** 45, 124 (1926).
[9] Ibid., **6,** 6 (1922); **10,** 50 (1927).
[10] Ibid., **14** (1931).

# XXIV. FISH AND OTHER MARINE PRODUCTS*

* See note at bottom of p. xv.

# XXV. FLAVORING EXTRACTS

## VANILLA EXTRACT AND ITS SUBSTITUTES

### 1 SPECIFIC GRAVITY*—OFFICIAL

Determine the specific gravity at 20/4° by means of a pycnometer, directed as under **XVII, 24.**

### 2 ALCOHOL—OFFICIAL

Proceed as directed in **10** and under **XVII, 59, 60** and **61.**

### 3 GLYCEROL—TENTATIVE

Proceed as directed under **XVII, 26, 27** and **28,** the method selected depending upon the quantity of sugar present, using such a quantity of the sample as contains 0.1–0.4 g of glycerol.

### VANILLIN AND COUMARIN (GRAVIMETRIC)[1]—OFFICIAL

(This method is not applicable to concentrated vanillin and coumarin preparations in which the quantity of vanillin and coumarin present in 50 cc exceeds the quantity dissolved by 100 cc of $H_2O$ at 20°. Then use a smaller quantity of the sample and dilute to 50 cc.)

### 4 PREPARATION OF SOLUTION

Measure 50 cc of the extract at 20° into a 250 cc beaker bearing marks showing volumes of 80 cc and 50 cc, dilute to 80 cc, and evaporate to 50 cc in a water bath kept at 70° or below. Dilute again with $H_2O$ to 80 cc and evaporate to 50 cc. Transfer to a 100 cc flask, rinsing the beaker with hot $H_2O$, add 25 cc of 8% neutral Pb-acetate soln, make up to the mark with $H_2O$, shake, and allow to stand 18 hours (overnight) at 37–40°. Decant into a small, dry filter, reserving the filtrate (Soln A) for the determination of vanillin and coumarin (**5**), the Pb number (**8** and **9**), and the residual color (**20.**)

### 5 DETERMINATION

(**a**) *Vanillin.*—Transfer a 50 cc aliquot of the filtrate (Soln A) to a separatory funnel and extract with 4 successive 15 cc portions of ether (previously washed twice with an equal volume of $H_2O$ to remove alcohol). Wash the combined ether solns 4 or 5 times with $NH_4OH$ (1+11), using 10 cc the first time and 5 cc thereafter. Reserve the ether soln for the determination of coumarin. Slightly acidify the combined ammoniacal solns with HCl (1+2), cool, and extract in a separatory funnel with 4 portions of washed ether, using about 40 cc altogether. Evaporate the ethereal solns at room temp., dry over $H_2SO_4$, and weigh. (The vanillin residue often appears first in the form of oil-like droplets, which on standing crystallize into light colored masses.) If, after standing in a desiccator, the residue is considerably discolored or gummy, extract the vanillin from it by treating with at least 15 successive portions of boiling petroleum ether (b. p. 40° or below); combine the petroleum ether extracts, evaporate to dryness, and weigh.

The residue, if pure vanillin, should be white crystals melting at approximately 80°. Dissolve a small quantity of the residue in 2 drops of HCl and add a crystal of resorcin. Vanillin gives a pink coloration.

(**b**) *Coumarin.*—Evaporate at room temp. the original ether extract obtained under (**a**) from which the vanillin has been removed by means of $NH_4OH$ (1+11), dry over $H_2SO_4$, and weigh.

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* See note 9, p. xvii.

The residue, if pure coumarin, melts at approximately 67°. Dissolve a small quantity of the residue in not more than 0.5 cc of hot $H_2O$ and add a few drops of 0.1 *N* I soln. Coumarin yields a brown precipitate which finally gathers in green flecks leaving a clear brown colored soln. The reaction is especially marked if the reagent is applied with a glass rod to a few drops of the soln on a white plate or tile.

### VANILLIN (COLORIMETRIC)[2]—OFFICIAL

**6** REAGENTS

(**a**) *Standard vanillin soln.*—Dissolve 0.1 g of pure vanillin in $H_2O$ and dilute to 1 liter.

(**b**) *Phosphotungstic-phosphomolybdic acid.*—To 100 g of pure Na-tungstate and 20 g of phosphomolybdic acid (free from nitrates and $NH_4$-salts), add 100 g of sirupy $H_3PO_4$ (containing 85% $H_3PO_4$) and 700 cc of $H_2O$. Boil over a free flame for 1½–2 hours, cool, filter, if necessary, and make up with $H_2O$ to 1 liter. An equivalent amount of pure molybdic acid may be substituted for the phosphomolybdic acid.

(**c**) *Sodium carbonate soln.*—Prepare 1 liter of a saturated soln of pure $Na_2CO_3$.

(**d**) *Lead soln.*—Dissolve 50 g each of basic and neutral Pb-acetate in $H_2O$ and make up to 1 liter.

**7** DETERMINATION

Transfer to a 100 cc volumetric flask a quantity of the sample that contains from 8–12 mg of vanillin (usually 5 cc). Add 75 cc of tap $H_2O$ at room temp. and 4 cc of the Pb soln. Dilute to 100 cc with $H_2O$ and mix. Filter thru a dry filter paper and pipet 5 cc of the clear filtrate into a 50 cc volumetric flask. Into another 50 cc volumetric flask pipet 5 cc of the standard vanillin soln. To each of these flasks add from a pipet 5 cc of Reagent (**b**), allowing the reagent to flow down the neck of the flask in such a way as to wash down the vanillin soln that may be on the sides of the flask. Mix the contents of the flasks by rotating and after 5 min. dilute contents to 50 cc with the $Na_2CO_3$ soln. Mix thoroly by inverting the flasks several times and allow to stand for at least 10 min. so that the precipitate that forms may separate completely. Filter the solns thru dry filter papers and compare the blue colors of the clear solns in a colorimeter. Report result as g of vanillin per 100 cc of extract.

### LEAD NUMBER

**8** *Method I. Winton*[3]*—Official*

To a 10 cc aliquot of the filtrate from the Pb-acetate precipitate obtained (Soln A, **4**) add 15 cc of $H_2O$, 10 cc of $H_2SO_4$ (1+9) and 100 cc of 95% alcohol and stir. Let stand overnight, filter thru a weighed Gooch crucible, wash the precipitate with 95% alcohol, dry at a moderate heat, ignite at low redness for 3 min., taking care to avoid the reducing flame, and weigh. Conduct a blank determination, using $H_2O$ containing 4 or 5 drops of glacial acetic acid in place of the sample. Calculate the Pb-number by the following formula and report as "Lead number—Winton":

$$P=\frac{100\times 0.6832\ (S-W)}{5}=13.66\ (S-W), \text{ in which}$$

$P$ = Pb-number (g of metallic Pb in the precipitate obtained from 100 cc of the sample);

$S$ = g of $PbSO_4$ corresponding to 2.5 cc of the Pb-acetate soln as determined in a blank analysis; and

$W$ = g of $PbSO_4$ obtained in 10 cc of the filtrate from the Pb-acetate precipitate, as obtained in **4**.

### *Method II. Wichmann*[4]*—Official*

### 9 REAGENT

*Lead acetate soln.*—Dissolve 80 g of neutral Pb-acetate in $H_2O$ that has been recently boiled, dilute to 1 liter, and filter if the soln is not clear.

### 10 DETERMINATION

Place 175 cc of boiled $H_2O$ in a round-bottomed flask of 1 liter capacity. Add by means of pipets 25 cc of the Pb-acetate soln and 50 cc of the sample. Place the flask in a hole in an asbestos board that is large enough to prevent the heating of the upper portion of the flask. (When the contents of the flask are reduced to 50 cc of liquid, the level of the liquid will be even with the top of the board, or slightly above it.) Connect the flask to a condenser, and with a moderate flame distil 200 cc into a volumetric flask. Calculate the approximate alcohol content of the extract from the specific gravity of the distillate. (For accurate results, redistil over alkali.) Transfer the residual soln to a 100 cc volumetric flask by means of $CO_2$-free $H_2O$ and a bent glass rod provided with a rubber tip. When cool, dilute to 100 cc with $CO_2$-free $H_2O$, mix, and filter thru a dry filter (Soln A).

Pipet 10 cc of Soln A into a 250 cc beaker, add 25 cc of $H_2O$, 2 cc of $H_2SO_4$ (1+1) and 100 cc of 95% alcohol, stir, and allow to settle overnight. Filter on a Gooch crucible, wash with 95% alcohol, dry, ignite at low redness, cool in a desiccator, and weigh. Conduct a blank determination, using 5 drops of glacial acetic acid in place of the sample and distil 150 cc instead of 200 cc. The difference between the two weights of $PbSO_4$ multiplied by 13.66 gives the Pb-number of the extract. Report as "Lead number—Wichmann."

### *Lead as Lead Chromate*[5]*—Official, first action*

### 11 REAGENT

*Potassium dichromate soln.*—Approximately 0.1 *N*. Dissolve 5 g of pure crystallized $K_2Cr_2O_7$ in $H_2O$ and dilute to 1 liter.

### DETERMINATION

Pipet 10 cc of the clear filtrate from the Pb precipitate (Soln A, **4** or **10**) to a 400 cc beaker and add 2 cc of glacial acetic acid, 25 cc of $H_2O$, and 25 cc of the $K_2Cr_2O_7$ soln. Heat the beaker and contents immediately with a moderate flame and continue heating until the precipitate changes in color from yellow to orange. Then filter the soln thru a weighed Gooch crucible provided with an asbestos mat and wash thoroly with hot $H_2O$ and then with a few cc each of alcohol and ether. Dry at 100°, cool in a desiccator, and weigh. Determine the Pb in the blank in the same manner. The difference in weights of $PbCrO_4$ multiplied by 12.82 is the Pb number.

### 12 TOTAL SOLIDS—OFFICIAL

Proceed as directed under **XXXIV, 4,** using 10 cc of the sample.

### 13 ASH—OFFICIAL

Evaporate 10 cc of the extract and determine the ash as directed under **XXVII, 8.**

### 14 ASH CONSTITUENTS

Proceed as directed under **XII.**

### 15 SUCROSE—OFFICIAL

Proceed as directed under **XXXIV, 22, 23,** or **28.**

## VANILLA RESINS

### 16 *Quantitative Test*[6]—*Tentative*

Pipet 50 cc of the extract into a small beaker, add 50 cc of $H_2O$, and evaporate to 50 cc on the steam bath. Add 50 cc of $H_2O$ and again evaporate to 50 cc. Cool. If the mixture has an acid reaction, add 2 cc of HCl (1+1). If the mixture is not acid to litmus, add HCl (1+1), dropwise, until distinctly acid to litmus paper, then 1 cc in excess. Cover and let stand overnight. Filter, wash 6 or 7 times with approximately 0.05 *N* HCl [9 cc of HCl (1+1) per liter of $H_2O$]. Dissolve the resin in warm 95% alcohol by pouring thru the filter. Evaporate the alcohol in a tared 50 cc beaker and dry to constant weight at 100°. Report results to 2 decimal places only. Reserve the resin for qualitative tests.

### 17 QUALITATIVE TEST—TENTATIVE

Place a portion of the dried residue in a few cc of 5% KOH soln. Vanilla resins dissolve, giving a deep red soln. Acidify, and a precipitate is obtained.

Dissolve a portion of the dried residue in 95% alcohol. To a portion of the soln add a few drops of a 10% $FeCl_3$ soln; to another portion add HCl. Neither produces any marked change in color if the residue consists of vanilla resins. Most other resins in alcoholic soln give color reactions with $FeCl_3$ or HCl.

To a portion of the filtrate obtained (**16**), add a few drops of basic Pb-acetate soln [**XXXIV, 18** (a)]. Owing to the excessive quantity of organic acids, gums, and other extractive matter, the precipitate is so bulky as almost to solidify. The filtrate from this precipitate should be almost colorless.

Test another portion of the filtrate from the resin for tannin with a soln of gelatin. Tannin is present in varying but small quantities, but should not be present in excessive quantities.

### 18 METHYL ALCOHOL—OFFICIAL

Proceed as directed under **XVII, 73, 74** or **75**, using the distillate from the determination of alcohol under **2**.

### 19 COLOR VALUE—TENTATIVE

Pipet 2 cc of the extract into a 50 cc volumetric flask and dilute to the mark with a mixture of equal parts of 95% alcohol and $H_2O$. Determine the color value of this diluted extract in terms of red and yellow by means of a Lovibond tintometer, using a 1-inch cell. To obtain the color value of the original extract multiply the figures for each color by 25.

### 20 RESIDUAL COLOR AFTER PRECIPITATION WITH LEAD ACETATE[7]—TENTATIVE

Determine the color value, in terms of red and yellow, of the filtrate from the Pb-acetate precipitate obtained under **4**, using a 1-inch Lovibond cell. Multiply the reading by 2 to reduce the results to the basis of the original extract. If the actual reading of the soln is greater than 5 red and 15 yellow, as may happen if the extract is highly colored with caramel, a half or quarter inch cell should be used, and the readings multiplied, respectively, by 4 or 8. To obtain the percentages of the two colors remaining in the Pb-acetate filtrate, divide the figures for red and yellow, respectively, by the corresponding figures of the original extract obtained under **19** and multiply the quotients by 100. Calculate also the ratio of red to yellow in both extract and Pb-acetate filtrate.

### 21 COLORS INSOLUBLE IN AMYL ALCOHOL—TENTATIVE

Proceed as directed under **XVII, 79**, using 25 cc of the extract and shaking with 25 cc of the Marsh reagent instead of 20 cc.

### 22 COLORING MATTERS OTHER THAN CARAMEL—TENTATIVE

Proceed as directed under **XXI.**

## LEMON AND ORANGE EXTRACTS

### 23 SPECIFIC GRAVITY*—OFFICIAL

Determine the specific gravity at 20/4° by means of a pycnometer, as directed under **XVII, 24.**

### ALCOHOL

### 24 *Method I—Official*

Pipet 50 cc of the extract, measured at 20°, into a 200 cc volumetric flask; dilute with $H_2O$ to approximately 200 cc; and allow the mixture to stand until the oil separates in a clear layer at the top, or centrifugalize and add $H_2O$ to bring the lower meniscus of the oil to the mark. Pour the mixture into a dry Erlenmeyer flask containing 5 g of light $MgCO_3$, stopper, shake well, and filter quickly thru a large, dry, folded filter. Introduce a 150 cc aliquot of the filtrate, measured at 20°, into a 300–500 cc distillation flask; attach the flask to a condenser; and distil almost 100 cc. Add $H_2O$ to complete the volume of the distillate to 100 cc at 20°, mix well, and determine the specific gravity at 20/4°. Ascertain the corresponding percentage of alcohol by volume from **XLII,** Tables **19-21,** and multiply the result thus obtained by $2\frac{2}{3}$ to obtain the percentage of alcohol by volume in the original sample.

### 25 *Method II*[8]*—Official*

(Applicable to extracts consisting only of oil, alcohol, and water)

Let $S$ represent the specific gravity of the extract at 20/4°, as determined under **23**; $O$, the specific gravity of the oil; and $p$, the percentage of oil found. Then $100-p$ will be the percentage of the water-alcohol soln, the specific gravity of which, represented by $P$, is calculated as follows:

$$S=\frac{Op+P\,(100-p)}{100},\ \text{whence}\ P=\frac{100\,S-Op}{100-P}.$$

The value of $E$, the alcohol equivalent of $P$, is obtained from **XLII,** Tables **19-21.** It gives the percentage of alcohol in the alcohol-water soln. To find the percentage of alcohol in the extract, apply the following formula:

$$\text{Percentage by volume of alcohol in the extract}=E\left(1-\frac{p}{100}\right).$$

The value of $O$ for lemon oil may be taken as 0.86 and for orange oil as 0.85.

### 26 GLYCEROL—TENTATIVE

Proceed as directed under **XVII, 26, 27** or **28,** the method selected depending upon the quantity of sugar present. Use a quantity of the sample that contains 0.1–0.4 g of glycerol.

### OILS OF LEMON AND ORANGE IN EXTRACTS

### 27 *I. Polarization—Official*

Without diluting, polarize the extract at 20° in a 200 mm tube. Divide the reading in degrees Ventzke by 3.2 in the case of lemon extract and by 5.2 in the case of orange extract; in the absence of other optically active substances, the result will be the percentage of oil by volume. If cane sugar is present, determine as directed under **35** and correct the reading accordingly. To obtain the percentage of oil by weight from the percentage by volume, multiply the volume percentage by 0.86 in the case of lemon extracts, and by 0.85 in the case of orange extracts, and divide the results by the specific gravity of the original extract.

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* See note 9, p. xvii.

### 28 *II. By Precipitation—Official*

Pipet 20 cc of the extract into a Babcock milk bottle. Add 1 cc of HCl (1+1), then 25–28 cc of $H_2O$ previously warmed to 60°; mix; and let stand in $H_2O$ at 60° for 5 min. Centrifugalize for 5 min.; fill the bottle with warm $H_2O$ to bring the oil into the graduated neck of the flask; again centrifugalize for 2 min., and place the flask in $H_2O$ at 60° for a few min. Note the percentage of oil by volume. If oil is present in amounts over 2%, add 0.4% to the percentage of oil noted to correct for the solubility of the oil. If less than 2% and more than 1% is present, add 0.3% for this correction. To obtain the percentage of oil by weight from the percentage by volume multiply the volume percentage by 0.86 in the case of lemon extracts, and by 0.85 in the case of orange extracts, and divide the result by the specific gravity of the original extract.

## TOTAL ALDEHYDES[9]—OFFICIAL

### 29 REAGENTS

(**a**) *Aldehyde-free alcohol.*—Allow 95% alcohol, containing 5 g of metaphenylenediamine hydrochloride per liter, to stand for at least 24 hours with frequent shaking. (Nothing is gained by previous treatment with KOH.) Boil under a reflux condenser for at least 8 hours, longer if necessary; allow to stand overnight; and distil, rejecting the first 10 and the last 5 cc which come over. Store in a dark, cool place in well-filled bottles. Twenty-five cc of this alcohol, on standing 20 min. at 14–16° with 20 cc of the sulfite-fuchsin soln should develop only a faint pink coloration. If a stronger color is developed, repeat the treatment with metaphenylenediamine hydrochloride as above.

(**b**) *Sulfite-fuchsin soln.*—Dissolve 0.5 g of fuchsin in 250 cc of $H_2O$, add an aqueous soln of $SO_2$ containing 16 g of the gas, allow to stand until colorless or nearly so, and make up to 1 liter with $H_2O$. Let stand 12 hours before using and keep in a refrigerator. This soln is liable to deteriorate and should be reasonably fresh when used.

(**c**) *Standard citral soln.*—Weigh 0.5 g of citral into a 50 cc volumetric flask, make up to mark with the aldehyde-free alcohol at room temp., stopper the flask, and mix by shaking. Dilute 10 cc of this soln with the aldehyde-free alcohol to 100 cc in a volumetric flask, stopper the flask, and mix by shaking. 1 cc of the dilute soln = 1 mg of citral.

### 30 DETERMINATION

Weigh approximately 25 g of the extract in a stoppered weighing flask, transfer to a 50 cc volumetric flask, and dilute to the mark at room temp. with aldehyde-free alcohol. Measure, at room temp., 2 cc (or other suitable quantity) of this soln into a comparison tube. Add 25 cc of the aldehyde-free alcohol (previously cooled to 14–16°), then 20 cc of the sulfite-fuchsin soln (also cooled) and finally make up to the 50 cc mark with aldehyde-free alcohol. Mix thoroly, stopper, and keep at 14–16° for 15 min. Prepare a standard for comparison at the same time and in the same manner, using 2 cc of the standard citral soln, and compare the colors developed. Calculate the amount of citral present and repeat the determination, using a quantity sufficient to give the sample approximately the strength of the standard. From this result calculate the quantity of citral in the sample. If the comparisons are made in Nessler tubes, standards containing 1, 1.5, 2, 2.5, 3, 3.5, and 4 mg of citral may be prepared and the trial comparison made against these, the final comparison being made with standards lying between 1.5 and 2.5 mg with 0.25 mg increments.

It is absolutely essential to keep the reagents and comparison tubes at the required temp., 14–16°. If the comparisons are made in a bath (this being possible only when the bath is of glass), the standards should be discarded within 25 min. after adding the sulfite-fuchsin soln. Give samples and standards identical treatment.

### CITRAL[10]—OFFICIAL, FIRST ACTION

(Lemon and orange extracts)

31

### REAGENTS

(a) *Metaphenylenediamine hydrochloride-oxalic acid soln.*—Dissolve 1 g of metaphenylenediamine hydrochloride in about 45 cc of 85% alcohol and 1 g of crystallized oxalic acid in a similar quantity of alcohol of the same strength and pour the two solns into a 100 cc volumetric flask. Add 2 or 3 g of fullers' earth, dilute to the mark with 85% alcohol, mix, and filter thru a double-folded filter.

(b) *Alcohol.*—95% by volume for lemon and orange extracts; 50–95% by volume for terpeneless lemon and orange extracts.

(c) *Standard citral soln.*—Dissolve 1 g of citral in alcohol, 90–95% by volume, dilute to 100 cc, and mix. Dilute 5 cc of this soln to 50 cc with alcohol, 90–95% by volume, and mix. 1 cc of the second soln = 1 mg of citral.

32

### DETERMINATION

Weigh 25 g of the extract into a 50 cc volumetric flask, dilute to the mark with alcohol, and mix. Pipet 2 cc or other suitable quantity of this soln into a colorimeter tube, add 10 cc of Reagent (a), dilute to suitable volume, and compare the resulting color with the colors of a set of standards containing known quantities of citral prepared like the sample.

33

### TOTAL SOLIDS—OFFICIAL

Proceed as directed under **XVII, 62,** using 10 cc of the sample measured at 20°.

34

### ASH—OFFICIAL

Ignite the residue from 10 cc of the extract as directed under **XXVII, 8.**

35

### SUCROSE—OFFICIAL

Neutralize the normal weight of the extract, evaporate to dryness, wash several times with ether, dissolve in $H_2O$, and determine as directed under **XXXIV, 22, 23** or **28.**

36

### METHYL ALCOHOL—OFFICIAL

Proceed as directed under **XVII, 73, 74** or **75,** using the distillate from the determination of alcohol under **24.**

37

### COLORING MATTERS—TENTATIVE

Proceed as directed under **XXI.**

38

### LEMON AND ORANGE PEEL COLOR—TENTATIVE

Place a few cc of the extract in each of 2 test tubes; to one, add slowly 3–4 volumes of HCl and to the other, several drops of $NH_4OH$. If the color is due to lemon or orange peel only, it is materially deepened by each treatment.

## LEMON AND ORANGE OILS

39

### SPECIFIC GRAVITY*—OFFICIAL

Determine the specific gravity at 20/4° by means of a pycnometer, as directed under **XVII, 24.**

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* See note 9, p. xvii.

### 40 INDEX OF REFRACTION—OFFICIAL

Determine the index of refraction with any standard instrument, making the reading at 20° (cf. **XXXI, 8**).

### 41 OPTICAL ROTATION—OFFICIAL

Determine the rotation at 20° with any standard instrument, using a 50 mm tube and Na-light. The results should be stated in angular degrees on a 100 mm basis. If instruments having the sugar scale are used, the reading for orange oils is above the range of the scale, but readings may be obtained by the use of standard levorotatory quartz plates, or by the use of a 25 mm tube. The true rotation cannot be obtained by diluting the oil with alcohol and correcting the rotation in proportion to the dilution.

### 42 TOTAL ALDEHYDES[11]—OFFICIAL

Weigh a small quantity of the sample into a small stoppered flask and dilute with aldehyde-free alcohol in the proportion of 2 g of lemon oil or 4 g of orange oil to 10 cc of soln. Determine the total aldehydes as directed under **30**, expressing the result as citral.

### *Kleber Method*[12]*—Official*

### 43 REAGENTS

(a) *Phenylhydrazine soln.*—Prepare a 10% soln in absolute alcohol. A sufficiently pure product can be obtained by distilling the commercial article in vacuo, rejecting the first portions coming over that contain $NH_3$.

(b) *Hydrochloric acid.*—0.5 *N*.

### 44 DETERMINATION

Weigh accurately about 15 g of the sample into a small, glass-stoppered flask, and add 10 cc of the phenylhydrazine soln. Allow to stand 30 min. at room temp. and titrate with the 0.5 *N* HCl, using either methyl or ethyl orange indicator. Titrate similarly 10 cc of the phenylhydrazine soln. The difference in the number of cc of 0.5 *N* acid used in these 2 titrations, multiplied by the factor 0.076, gives the weight of citral in the sample. If difficulty is experienced in detecting the end point of the reaction, titrate until the soln is distinctly acid; transfer to a separatory funnel; and draw off the alcoholic portion. Wash the oil with $H_2O$, adding the washings to the alcoholic soln; titrate back with 0.5 *N* alkali; and make the necessary corrections.

### 45 *Hiltner Method*[12]*—Official*

Weigh accurately about 2 g of lemon oil or 8 g of orange oil into a 100 cc volumetric flask, dilute to the mark with 95% alcohol, and proceed as directed under **32**, using 2 cc of the dilute soln for the comparison.

### 46 PHYSICAL CONSTANTS OF THE 10 PER CENT DISTILLATE[13]—OFFICIAL

Place 50 cc of the sample in a 3-bulb Ladenburg flask having the main bulb 6 cm in diameter and of 120 cc capacity and the condensing bulbs of the following dimensions: 3.5 cm, 3 cm, and 2.5 cm. The distance from the bottom of the flask to the opening of the side arm should be 20 cm. Distil the oil at the rate of 2 cc per min. until 5 cc has been distilled. Determine the refractive index and rotation of this distillate as directed under **40** and **41**.

### 47 PINENE[14]—OFFICIAL

Mix the 10% distillate obtained under **46** with 5 cc of glacial acetic acid, cool the mixture thoroly in a freezing bath, and add 10 cc of ethyl nitrite. Then add slowly, with constant stirring, 2 cc of HCl (2+1). Keep the mixture in the freezing bath 15 min. Collect the crystals formed on a filter, using suction, and wash with 95% alcohol. Return the combined filtrate and washings to the freezing bath for 15 min. Collect the additional crystals formed on the original filter. Wash the combined crops of crystals thoroly with alcohol. Dry at room temp. and dissolve in a minimum quantity of $CHCl_3$. Add methyl alcohol to the $CHCl_3$ soln, a little at a time, until the nitroso-chlorides crystallize out. Mount the separated and dried crystals in olive oil and examine under the microscope. Pinene nitroso-chloride crystals have irregular pyramidal ends, while limonene nitroso-chloride crystallizes in needles.

## ALMOND EXTRACT

**48**

### ALCOHOL—TENTATIVE

As almond extract usually contains only about 1% of almond oil, in most cases the alcohol can be calculated from the specific gravity of the extract. If the extract is high in solids, determine the alcohol as follows: Add 25 cc of the extract, measured at 20°, to 75 cc of saturated NaCl soln in a separatory funnel and extract twice with 50 cc portions of petroleum ether (b.p. 40–60°). Collect the petroleum ether extract in a second separatory funnel, and wash twice with 2 portions (25 cc) of saturated brine. Combine the original salt soln with the washings, add a little powdered pumice, and distil into a 100 cc volumetric flask. When almost 100 cc has been distilled, make up to the mark with $H_2O$ at 20° and determine alcohol from the specific gravity, as directed under **XVII, 61.**

### BENZALDEHYDE—TENTATIVE

**49**

#### REAGENT

*Phenylhydrazine soln.*—Add 3 cc of glacial acetic acid to 40 cc of $H_2O$ and mix with 2 cc of phenylhydrazine.

**50**

#### DETERMINATION

Measure out 2 portions of 10 cc each of the extract into 300 cc Erlenmeyer flasks and add 10 cc of the phenylhydrazine soln to one flask and 15 cc to the other. Allow the mixtures to stand overnight in a dark place. Then add 200 cc of $H_2O$, and filter thru a weighed Gooch crucible provided with a thin layer of asbestos. Wash the precipitate first with cold $H_2O$ and finally with 10 cc of 10% alcohol. Dry at 70° for 3 hours at a pressure not to exceed 100 mm of Hg or to constant weight over $H_2SO_4$. The weight of the precipitate, multiplied by the factor 5.408, gives the weight of benzaldehyde in 100 cc of the sample. If duplicate determinations do not agree, repeat the operation, using a larger quantity of the phenylhydrazine soln.

**51**

### BENZOIC ACID[15]—TENTATIVE

Measure 10 cc of the extract into a 100 cc flask and add 10 cc of a 10% NaOH soln and 20 cc of 3% $H_2O_2$ soln; cover with a watch-glass; and place in a water oven. Oxidation of the aldehyde to benzoic acid begins almost immediately and should be continued 5–10 min. after all odor of benzaldehyde has disappeared (usually 20–30 min.). Remove the flask from the water oven; transfer the contents to a separatory funnel, rinsing off the watch-glass; add 10 cc of $H_2SO_4$ (1+5); and cool the contents of the funnel to room temp. under the water tap. Extract the benzoic acid with 4 portions of 25, 25, 20, and 20 cc of ether, respectively, and wash the combined extracts with 2 portions of 5–10 cc of $H_2O$, or until all $H_2SO_4$ is removed. Filter into a weighed dish, evaporate at room temp., dry overnight in a desiccator, and weigh the benzoic acid. Multiply the result by 10.

Multiply the grams per 100 cc of benzaldehyde obtained under **50** by 1.151 to obtain the equivalent of benzoic acid and subtract this product from the grams per 100 cc of total benzoic acid obtained above. The difference is the grams of benzoic acid per 100 cc of the extract.

### HYDROCYANIC ACID

**52** *Qualitative Test—Tentative*

Add several drops of a freshly prepared 3% $FeSO_4$ soln and a single drop of 1% $FeCl_3$ soln to several cc of the extract. Mix thoroly and add 10% NaOH soln, dropwise, until no further precipitate forms and then $H_2SO_4$ (1+9) to dissolve the precipitate. In the presence of even small quantities of HCN, a Prussian blue coloration or suspension will develop.

**53** *Quantitative Method—Tentative*

(In the absence of chlorides)

Measure 25 cc of the extract into a small flask and add 5 cc of freshly precipitated $Mg(OH)_2$ (Cl-free). Titrate with 0.1 *N* $AgNO_3$ soln, using $K_2CrO_4$ as an indicator. 1 cc of 0.1 *N* $AgNO_3$ = 0.0027 g of HCN.

### NITROBENZOL

**54** *Qualitative Test—Tentative*

Boil a few cc of the extract with some Zn dust and acetic acid and filter. Add to the filtrate a drop of $CHCl_3$, make strongly alkaline with 10% NaOH soln, and heat. The presence of nitrobenzol in the original extract is indicated by the development of the characteristic odor of phenylisonitrile.

## CASSIA, CINNAMON, AND CLOVE EXTRACTS

**55** ALCOHOL—TENTATIVE

Determine as directed under **48**.

**56** OIL[15]—TENTATIVE

Transfer 10 cc of the extract to a separatory funnel, add 30 cc of $H_2O$, acidify with 1 cc of HCl (1+1); and extract 3 times with ether, using not less than 100 cc altogether. Wash the combined ether solns twice with $H_2O$, and in the case of cinnamon extract dry by shaking with a small quantity of granulated $CaCl_2$. Transfer to a weighed wide-mouthed weighing bottle and evaporate the ether as rapidly as possible on a boiling water bath, rotating the liquid upon the sides of the bottle in order to rid the residual oil of traces of ether. Weigh the residue and divide the weight by the specific gravity of the oil in order to obtain the percentage of oil by volume. In the case of clove oil, allow the weighing bottle to remain in the balance case until the usual film of moisture has evaporated. The time of weighing, however, should not be delayed over 3 min. Determine the refractive index of the residual oils at 20°. Dissolve a drop of the oil in several drops of alcohol and add a drop of 10% $FeCl_3$ soln.

The following tabulation gives the specific gravity, refractive index at 20°, and color reaction with $FeCl_3$ soln:

| OIL | SPECIFIC GRAVITY | REFRACTIVE INDEX AT 20° | COLOR REACTION WITH FERRIC CHLORIDE SOLUTION |
|---|---|---|---|
| Cassia | 1.05 | 1.585–1.600 | Brown |
| Cinnamon | 1.03 | 1.590–1.599 | Green |
| Cloves | 1.055 | 1.560–1.565 | Deep blue |

## GINGER EXTRACT

57 ALCOHOL—TENTATIVE

Determine as directed under **XVII, 61.**

58 SOLIDS—TENTATIVE

Evaporate 10 cc of the extract nearly to dryness on the steam bath, dry for 2 hours in a water oven at the temp. of boiling $H_2O$, and weigh.

59 GINGER (QUALITATIVE TEST)—TENTATIVE

Dilute 10 cc of the extract to 30 cc, evaporate to 20 cc, decant into a separatory funnel, and extract with an equal volume of ether. Allow the ether to evaporate spontaneously in a porcelain dish, and to the residue add 5 cc of 75% $H_2SO_4$ and about 5 mg of vanillin. Allow to stand 15 min. and add an equal volume of $H_2O$. In the presence of ginger extract an azure blue color develops.

60 CAPSICUM (QUALITATIVE TEST)—TENTATIVE

To 10 cc of the extract add cautiously NaOH soln (1+9) until the soln reacts very slightly alkaline with litmus paper. Evaporate at about 70° to approximately $\frac{1}{4}$ the original volume and render slightly acid with $H_2SO_4$ (1+9), testing with litmus paper. Transfer to a separatory funnel, rinsing the dish with $H_2O$, and extract with an equal volume of ether, avoiding the formation of an emulsion by shaking the funnel gently 1–2 min. Draw off the lower layer and wash the ether extract once with about 10 cc of $H_2O$. Transfer the washed ether extract to a small evaporating dish, render decidedly alkaline with 0.5 *N* alcoholic KOH, and evaporate at about 70° until the residue is pasty. Then add about 20 cc more of the 0.5 *N* alcoholic KOH and allow to stand on the steam bath until the gingerol is completely saponified (about 30 min.). Dissolve the residue in a little $H_2O$ and transfer with $H_2O$ to a small separatory funnel. The volume should not exceed 50 cc. Extract the alkaline soln with an equal volume of ether. Wash the ether extract repeatedly with small quantities of $H_2O$ until no longer alkaline to litmus. Transfer the washed extract to a small evaporating dish and allow the ether to evaporate spontaneously. Finally test the residue for capsicum by moistening the tip of the finger, rubbing it on the bottom and sides of the dish, and then applying the finger to the end of the tongue. A hot, stinging, or prickly sensation, which persists for several min., indicates capsicum or other foreign pungent substances.

## PEPPERMINT, SPEARMINT, AND WINTERGREEN EXTRACTS

61 ALCOHOL—TENTATIVE

Proceed as directed under **48.**

62 OIL[16]—TENTATIVE

Pipet 10 cc of the extract into a Babcock milk bottle, add 1 cc of $CS_2$, mix thoroly and then add 25 cc of cold $H_2O$ and 1 cc of HCl. Close the mouth of the bottle and shake vigorously; centrifugalize for 6 min., and remove all but 3–4 cc of the supernatant liquid, which should be practically clear, by aspirating thru a glass tube of small bore. Connect the stem of the bottle with a filter pump, immerse the bottle in $H_2O$ kept at approximately 70° for 3 min., remove from the bath every 15 seconds, and shake vigorously. Continue in the same manner for 45 seconds, using a boiling water bath. Remove from the bath and shake while cooling. Disconnect from the suction and fill the bottle to the neck with saturated salt soln at room temp., centrifugalize for 2 min., and read the volume of the separated oil from the top of the meniscus. Multiply the reading by 2 to obtain the percentage of oil by volume. In the case of wintergreen, use as a floating medium a mixture of 1 volume of concentrated $H_2SO_4$ and 3 of saturated $Na_2SO_4$ soln.

### 63 METHYL SALICYLATE IN WINTERGREEN EXTRACT[15]—TENTATIVE

Mix 10 cc of the extract with 10 cc of 10% KOH soln. Heat on a steam bath until the volume is reduced about one-half. Add a distinct excess of dilute HCl (1+1); cool; and extract with 3 portions of ether, 40, 30, and 20 cc, respectively. Filter the extract thru a dry filter into a weighed dish, wash the paper with 10 cc of ether, and allow the filtrate and washings to evaporate spontaneously. Dry in a desiccator containing $H_2SO_4$ and weigh. Multiply the weight of salicylic acid so found by 9.33 to obtain the percentage by volume of methyl salicylate in the sample.

## ANISE AND NUTMEG EXTRACTS

### 64 OIL[15]—TENTATIVE

To 10 cc of the extract in a Babcock milk bottle, add 1 cc of HCl (1+1), then sufficient half-saturated salt soln, previously heated to 60°, to fill the flask nearly to the neck. Cork and let stand in $H_2O$ at 60° for about 15 min., rotate occasionally, and centrifugalize for 10 min. at about 800 r.p.m. Add brine till the oil rises into the neck of the bottle and again centrifugalize for 10 min. If the separation is not satisfactory or the liquid is not clear, cool to about 10° and centrifugalize for an additional 10 min. Multiply the reading by 2 to obtain the percentage of oil by volume.

## OILS OF LEMON, ORANGE, AND LIMES IN VEGETABLE AND MINERAL OILS

### *I. By Steam Distillation*[17]*—Official*

### 65 APPARATUS

(a) *Steam generator filled with $H_2O$.*—An oil can holding 1 gallon will serve the purpose.

(b) *Distillation flask.*—A Kjeldahl flask of about 750 cc capacity, with shortened neck, about 10 inches in height over all.

(c) *Spray tube.*—A glass tube with a small perforated bulb at the end passes thru a rubber stopper and reaches to the bottom of the distillation flask.

(d) *Bent glass tube.*—About 8 mm in diameter. Connects distillation flask to upright condenser. The shape of this tube allows the vapor condensing in the tube to return to the distillation flask.

(e) *Liebig condenser.*—With 20-inch water jacket.

(f) *Wilson receiving flask.*—Shaped like a Babcock test bottle with a graduated neck but of much larger capacity and with a vertical glass outlet tube sealed on near the bottom. The upper end of the outlet tube is turned down. The capacity of the flask is about 250 cc. The neck may consist of a portion of a buret graduated from 0–25 cc with top flared out. The outlet tube is about 3 mm in diameter, and the end is at such a height that when the flask is filled with $H_2O$ the meniscus in the neck will be between the 0 and 1 cc marks.

### 66 DETERMINATION

Measure 100 cc of the sample in a graduated cylinder and transfer to the distillation flask. Immerse the flask in a water bath and connect with the condenser by means of the bent glass tube. Fill the receiving flask with $H_2O$ and place under the condenser in such a way that the end of the condenser will be about 0.5 inch above the level of the $H_2O$ in the receiving flask. Place a 200 cc graduated cylinder under the end of the outlet tube to catch the displaced liquid. Heat the water bath to boiling and pass steam thru the sample until 200 cc of liquid has been collected in the graduated cylinder.

Disconnect the apparatus, allow the receiving flask to stand for 15 min., or until separation of oil is complete, and read the volume of oil obtained. Calculate the percentage (by volume) of essential oil in the sample by dividing the reading by 0.90 for lemon oil in corn and cottonseed oils, 0.95 for orange oil in corn and cottonseed oils, and by 0.78 for distilled or expressed oil of limes in corn and cottonseed oils. Where the menstruum is mineral oil, subtract 0.3 cc from the reading before dividing by the factors 0.90, 0.95, and 0.78 for lemon oil, orange oil, and oil of limes, respectively.

### 67 *II. By Polarization*[18]*—Tentative*

Polarize the sample at 20° in a 200 mm tube, making 5 readings. From the average of these readings in degrees Ventzke subtract, for corn oil +0.6°, for cottonseed oil −0.3°, for peanut oil +0.2°, and for mineral oil +5.5°, as a correction for the rotatory effect of the menstruum. To obtain the percentage by volume of the essential oil in the mixture, divide the corrected polariscopic reading so obtained by the factor 3.4 for lemon oil in corn oil, 3.7 for lemon oil in cottonseed oil, 3.6 for lemon oil in peanut oil, 3.5 for lemon oil in mineral oil, 5.4 for orange oil in corn oil, 5.7 for orange oil in cottonseed oil, 5.6 for orange oil in mineral oil, 2.0 for oil of limes in corn oil, 2.3 for oil of limes in cottonseed oil, and 2.2 for oil of limes in mineral oil.

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# XXVI. FRUITS AND FRUIT PRODUCTS

## 1 PREPARATION OF SAMPLE—OFFICIAL

Transfer all samples received in open packages (i.e., not in sterile condition) without delay to glass-stoppered containers and keep in a cool place. Make the determinations of alcohol, total and volatile acids, solids, and sugars, particularly in the case of fruit juices and fresh fruits, at once, as fermentation is liable to begin very soon. (Portions for the determination of sucrose and reducing sugars may be weighed and kept for several days without fermenting if the slight excess of neutral Pb-acetate soln required in the determination is added.) The various products are prepared for analysis as follows:

**(a)** *Juices.*—Mix thoroly by shaking to insure uniformity in sampling and filter thru muslin previously washed and dried. Prepare fresh juices by pressing the well-pulped fruit in a jelly bag and filtering thru muslin previously washed and dried. Express the juice of citrus fruit by means of one of the common devices for squeezing oranges or lemons and strain the expressed juice thru muslin previously washed and dried.

**(b)** *Jellies and sirups.*—Mix thoroly to insure uniformity in sampling.

**($b_1$)** *Preparation of soln.*—Weigh 300 g of the thoroly mixed sample into a 2 liter flask and dissolve in $H_2O$, heating on a steam bath, if necessary. Apply as little heat as possible to minimize inversion of the sucrose. Cool, dilute to the mark, mix thoroly by shaking, and use aliquots for the various determinations. If insoluble material is present, mix thoroly and filter before taking the aliquots.

**(c)** *Fresh fruits, dried fruits, canned fruits, preserves, jams, and marmalades.*—Pulp by grinding in a large mortar or by passing thru a food chopper and mix thoroly, completing the operation as quickly as possible to avoid loss of moisture. In the case of dried fruits, pass the sample thru the food chopper three times, mixing thoroly after each grinding. In the case of stone fruits, remove the pits, and determine their proportion in a weighed sample. In the case of canned fruits, an examination of the sirup in which the fruits are preserved is often sufficient. Separate the liquor by draining (cf. **XXXV, 2**) and treat as directed under (**a**).

**($c_1$)** *Preparation of soln.*—Weigh 300 g of the well-pulped and mixed sample into a 1.5-2-liter beaker; add about 800 cc of $H_2O$; and boil 1 hour, replacing at intervals the $H_2O$ lost by evaporation. Transfer to a 2 liter volumetric flask, cool, dilute to volume, and filter. With unsweetened fruit it is desirable, tho not actually necessary, that sugar be added before boiling; therefore weigh 150 g of fruit, add 150 g of sugar and 800 cc of $H_2O$ and proceed as directed above.

## 2 ALCOHOL—OFFICIAL

Determine alcohol in 50 g of the original material as directed under **XXXIV, 79.**

## 3 MOISTURE

### DRIED FRUITS (GENERAL)—OFFICAL

Spread 5–10 g of the sample, prepared as directed under **1** (**c**), as evenly as possible over the bottom of a metal dish approximately 8.5 cm in diameter and provided with a tightly fitted cover; weigh; and dry at 70° for 12 hours under a pressure not to exceed 100 mm of Hg. During the drying admit to the oven a slow current of air, about 2 bubbles per second, dried by passing thru concentrated $H_2SO_4$. (The metal dish must be placed in direct contact with the metal shelf of the oven.) Replace the cover, cool in a desiccator, and weigh. Disregard any temporary drop of oven temp. that may occur during the early part of the drying period owing to rapid evaporation of $H_2O$. With raisins and fruit similarly rich in sugar, use about 5 g of sample and dry and weigh with the dish about 2 g of finely divided asbestos. Moisten with hot $H_2O$, mix the sample and asbestos thoroly, evaporate on a steam bath barely to dryness, and complete drying as directed above.

### 4 DRIED APPLES[1]—TENTATIVE

(A rapid method applicable only to dried apples)

Spread 5–10 g of the sample, prepared as directed under **1** (**c**), as evenly as possible over the bottom of a metal dish approximately 8.5 cm in diameter and provided with a tightly fitted cover; weigh; and dry for 4 hours in an oven at the temp. of boiling $H_2O$. Replace the cover, cool in a desiccator, and weigh. Place the dish on one of the shelves—not on the oven bottom. Provide a vent in the top of the oven to insure ventilation.

## TOTAL SOLIDS

### 5 FRESH AND CANNED FRUITS, JAMS, MARMALADES AND PRESERVES—OFFICIAL

(Insoluble matter present)

Weigh accurately 20 g of pulped fresh fruit, or a quantity of fruit products that will give not more than 3–4 g of dry material, into a large flat-bottomed dish. If necessary to secure a thin layer of the material, add a few cc of $H_2O$ and mix thoroly. Dry at 70° under a pressure of not to exceed 100 mm of Hg until consecutive weighings made at intervals of 2 hours do not vary more than 3 mg.

### 6 FRUIT JUICES, JELLIES AND SIRUPS—OFFICAL

(No insoluble matter present)

Proceed as directed under **XXXIV, 3, 5, 6** or **7,** using the sample prepared as directed under **1** (**a**) or (**b**).

### 7 WATER-INSOLUBLE SOLIDS[2]—TENTATIVE

Weigh 25 g of the sample, prepared as directed under **1** (**c**), into a 400 cc beaker; add 200 cc of $H_2O$; cover; heat to boiling; and boil vigorously for 30 min., replacing at intervals the $H_2O$ lost by evaporation. Prepare about 0.5 g of absorbent cotton or a coarse, qualitative 15 cm filter paper by drying in a water oven and weighing in a flat-bottomed dish provided with a cover. Filter the soln thru the absorbent cotton or filter paper and wash the residue and filter with hot $H_2O$ until the wash $H_2O$ is colorless and no longer acid to litmus paper. Transfer the cotton or paper filter containing the insoluble solids to the flat-bottomed dish, dry to constant weight in a water oven, and weigh. The increased weight represents the water-insoluble solids in the sample taken. The filter of absorbent cotton may be prepared by forming it into the shape of the funnel and by means of a wire or glass rod forcing a pledget of the cotton into the stem, but leaving the portion against the sides loose.

### 8 TOTAL ASH—OFFICIAL

Proceed as directed under **XXVII, 8,** the temp. of ashing not to exceed 525°, using 25 g of juices, fresh fruits, and canned fruits and 10 g of jellies, sirups, preserves, jams, marmalades, and dried fruits.

If the ash of the water-soluble portion only is desired, evaporate to dryness 100 cc of the soln prepared as directed under either **1** ($b_1$) or **1** ($c_1$) and proceed as directed under **XXVII, 8.**

### 9 ALKALINITY OF THE ASH—OFFICIAL

Into the Pt dish containing the ash obtained under **8** introduce a measured excess of 0.1 *N* HCl, warm on a steam bath, cool, add a few drops of methyl orange indicator, and titrate the excess acid with 0.1 *N* NaOH soln. Report the result as alkalinity, the number of cc of 0.1 *N* acid required to neutralize the ash from 100 g of sample, and as alkalinity number, the number of cc of *N* acid required to neutralize 1 g of ash. Reserve the filtrate for the determination of S in ash.

### 10 SULFUR IN ASH—OFFICIAL

(For products containing a basic ash)

Add 5 cc of HCl (1+2.5) to the soln remaining after the determination of alkalinity of ash under **9** and evaporate to dryness. Heat to 110° for 1 hour to dehydrate any $SiO_2$. Take up in 5 cc of the dilute HCl and filter, washing the filter paper well with hot $H_2O$. Heat the filtrate to boiling and add dropwise from a buret or pipet 5 cc of 10% $BaCl_2$ soln. Evaporate to 100 cc and let stand overnight. Filter on a weighed Gooch or Munroe crucible or on a 7 cm ashless filter paper, wash with hot $H_2O$ until the filtrate is free from chlorides, dry, ignite over a Bunsen burner, and weigh as $BaSO_4$. As the quantity of precipitate is small, exercise great care and make the determination in duplicate. Report the result as mg of S per 100 g and as percentage of S in ash.

### 11 TOTAL SULFUR[2]—TENTATIVE

(For sulfured products and for samples containing little ash or an acidic ash)

In a casserole as large as can be placed in the electric muffle furnace available, place 1–3 g of MgO (1 g for fruit juices, 3 g for heavily sugared products and for dried fruits—or an equivalent quantity of $Mg(NO_3)_2$), 1 g of powdered sucrose, and 50 cc of $HNO_3$. Then add 5–10 g of the sample prepared as directed under **1** (**a**), (**b**), or (**c**). Place the same quantities of the reagents in another casserole for a blank. Evaporate on a steam bath to a pasty consistency. Place the casserole in a cold electric muffle and gradually heat to not above dull redness until all $N_2O_4$ fumes have been driven off. (All organic matter will have been destroyed.) Cool, dissolve in HCl (1+2.5), and filter. Adjust the acidity so that the soln contains 0.5–1 g of free HCl, heat to boiling, and add dropwise 5 cc of a 10% $BaCl_2$ soln. Evaporate to 100 cc, allow to stand overnight, filter, wash, ignite, and weigh the $BaSO_4$. Correct the result for the $BaSO_4$ obtained in the blank and report as mg of S per 100 g. The determination should be made in a room free from S fumes.

## CHLORINE IN ASH

### 12 *Method I—Official**

### 13 *Method II—Tentative*

Proceed as directed under **XII, 33** and **35.**

## POTASSIUM—TENTATIVE

### 14 REAGENTS

(**a**) *Ammonium chloride soln.*—Dissolve 100 g of $NH_4Cl$ in 500 cc of $H_2O$, add 5–10 g of pulverized $K_2PtCl_6$, and shake at intervals for 6–8 hours. Allow the mixture to settle overnight and filter. (The residue may be used for the preparation of a fresh supply.)

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* See note 7, p. xvii.

(**b**) *Platinum soln.*—For materials containing less than 15% of $K_2O$, a $PtCl_4$ soln containing 0.2 g of metallic Pt (0.42 g of $H_2PtCl_6$) in each 10 cc is recommended.

(**c**) *Alcohol.*—90%. Sp. gr. 0.8339 at 15.6/15.6°.

### 15 PREPARATION OF SOLUTION

(A) Dissolve the ash in HCl. If it is desired to take an aliquot, filter in a volumetric flask, wash the filter thoroly, and make up to volume. Pipe an aliquot into a beaker, adjust to a volume of 50–75 cc, heat to boiling, and add a slight excess of $NH_4OH$ and then sufficient saturated $NH_4$-oxalate soln to precipitate all the lime and Al present. Filter into a large Pt dish and wash the filter thoroly.

### 16 DETERMINATION

Evaporate the soln from (A) nearly to dryness, add 1 cc of $H_2SO_4$ (1+1), evaporate to dryness, and ignite to whiteness. Maintain a full red heat until the residue is perfectly white. Dissolve the residue in hot $H_2O$, using at least 20 cc for each dg of $K_2O$ present; add a few drops of HCl and then an excess of Reagent (**b**). Evaporate on a water bath to a thick paste, avoiding exposure to $NH_3$.Treat the residue with 90% alcohol. Filter on a dry tared Gooch crucible with an asbestos mat that has been washed thoroly with 90% alcohol and dried at 100° for 30 min. Wash the precipitate thoroly with 90% alcohol, both by decantation and on the crucible mat, and continue the washings after the filtrate is colorless, using about 200 cc of wash solns. Then wash 5 or 6 times with 10 cc portions of the $NH_4Cl$ soln to remove impurities from the precipitate. Wash again with four or five 10 cc portions of 90% alcohol and dry the precipitate for 30 min. at 100°. Weigh, wash again with several 100 cc portions of 90% alcohol, dry, and reweigh until a constant weight of $K_2PtCl_6$ is obtained. Calculate to $K_2O$. The precipitate should be completely soluble in $H_2O$.

## MANGANESE—TENTATIVE

### 17 REAGENTS

(**a**) *Sodium hydroxide soln.*—10%, freshly prepared.
(**b**) *Sodium oxalate soln.*—Saturated.
(**c**) *Oxalic acid soln.*—3%.
(**d**) *Sodium acetate soln.*—20%.
(**e**) *Sodium dihydrogen phosphate.*—10%.
(**f**) *Ammonium hydroxide soln.*—100 cc of $NH_4OH$ diluted to 1 liter.
(**g**) *Hydrochloric acid.*—5 cc of HCl diluted to 30 cc.
(**h**) *Ammonium oxalate soln.*—Saturated.

### 18 PREPARATION OF SOLUTION

(B) Dissolve the ash in HCl, evaporate to dryness, and bake at 110° for 1 hour to dehydrate any $SiO_2$. Dissolve the residue in the dilute HCl and filter into a volumetric flask. Wash the filter thoroly and make up to volume.

### 19 DETERMINATION

To an aliquot of soln B add sufficient Br water to oxidize any ferrous Fe to the ferric state. Boil off the excess Br. Dilute to 150 cc and heat to boiling. Add sufficient $NaH_2PO_4$ (**e**) to combine with all the Fe and Al present. Add plenty of bromcresol green indicator, and while the mixture is gently boiling add NaOH (**a**) dropwise to the first permanent turbidity or an initial color change in the event no Fe or Al compounds are present. Continue neutralization by slowly adding Na-acetate (**d**) to give a yellow-green color. Fe- and Al-phosphates are completely precipitated at a pH of 4, at which point bromcresol green indicator is yellow-green (see color chart in Clark's "The Determination of Hydrogen Ions"). Boil gently for 1–2 min. if any precipitate of Al- or Fe-phosphate forms. Allow to settle, filter, wash carefully, and discard the precipitate. To the filtrate add 10 cc of Na-acetate (**d**) and adjust the pH to 4.2–4.4 (indicated by a yellow-green color with bromcresol green indicator) by adding dilute HCl (**g**) dropwise. Add sufficient Br $H_2O$ to color the soln distinctly orange, cover with a watch-glass, and boil gently for about 3 min. Take great care to avoid bumping. Allow the mixture to settle, add a little more Br $H_2O$, and again boil gently for 1–2 min. Again allow to settle, filter, and wash beaker and filter thoroly. The filtrate is reserved for Ca and Mg determinations. The Mn is determined colorimetrically by the periodate method. Dissolve the hydrated oxide precipitate from the filter into the original beaker with as little soln of $H_2O$ saturated with $SO_2$ as possible. Wash the filter paper thoroly with hot $H_2O$. Boil to remove all odor of $SO_2$, add 10 cc of $H_2SO_4$ and 10–20 cc of $HNO_3$, carefully dilute to 50–75 cc and heat to boiling, slowly introducing small quantities of $KIO_4$ (about 0.05 g) with a spatula until a maximum color is produced. (About 0.2 g of periodate is sufficient.) Cool, and introduce into a volumetric flask. The amount of Mn in the final dilution for colorimetric comparison should be no more than 1 mg per 50 cc. Compare the color with standards prepared as in **XII, 14,** except to substitute 10 cc of $HNO_3$ for the $Fe(NO_3)_2$. Very accurate results may be expected. Report as percentage of $Mn_3O_4$ by multiplying $KMnO_4$ by the factor 0.4827.

## CALCIUM[3]—TENTATIVE

### 20 *I. Double Precipitation Method*

Evaporate the filtrate from the Mn determination to 100–150 cc. Boil off any Br remaining and adjust the pH to 4.4–4.6 (green to green-blue with bromcresol green indicator) by adding 20% Na-acetate (**d**). (A pH of 4.4–4.6 is the most favorable for precipitation of Ca-oxalate.) Add sufficient saturated Na-oxalate soln (**b**) dropwise to precipitate all the Ca from the boiling soln, and continue to boil until the oxalate begins to settle, or digest for 15 min. on the steam bath. Allow to settle until clear, filter, and wash the precipitate thoroly with hot $H_2O$. Reserve the filtrate and washings for the Mg determination. Carefully wash the precipitate back into the original beaker, heat, and dissolve the oxalate by adding as little HCl as possible. Reprecipitate the Ca by adding dilute $NH_4OH$ dropwise until the pH is again 4.4–4.6 (green to green-blue with bromcresol green indicator). Add a slight excess of saturated $NH_4$-oxalate soln (**h**) while still hot. Digest on a steam bath for 1 hour and set aside until the supernatant liquid is clear, preferably overnight. Filter, and wash with hot $H_2O$. Determine the Ca either gravimetrically or volumetrically by the usual methods. (For small quantities of Ca the gravimetric method is preferred.) Report as CaO.

If Mg is not to be determined, precipitate the Ca once from the boiling soln freed from Fe, Al, and Mn with saturated $NH_4$-oxalate soln (**h**), digest, and determine as described.

### 21 *II. Single Precipitation Method*[4]

Evaporate the filtrate and washings from the Mn determination to 200–250 cc. Add 8-10 drops of bromcresol green indicator and sufficient Na-acetate (**d**) to change the pH to 4.8–5.0 (blue). Cover with a watch-glass and heat to boiling. Precipitate the Ca slowly by adding oxalic acid soln (**c**), a drop every 3–5 seconds, until the pH is changed back to 4.4–4.6 (the optimum for Ca-oxalate precipitation) as indicated by the appearance of a distinct green shade. A change of color will indicate an excess of oxalic acid—more would develop yellow tints, showing an undesirable displacement of the pH. Boil 1–2 min. and allow to settle until clear. Filter, and wash thoroly with hot $H_2O$. Determine either gravimetrically or volumetrically as in the double precipitation method.

### 22 MAGNESIUM—TENTATIVE

Add 2–3 drops of HCl to the filtrate and washings from the Ca determination and evaporate to 75–100 cc. If the quantity of phosphates naturally in the sample, or added for the purpose of precipitating Fe and Al, is insufficient to precipitate all the Mg expected, more must be added but a large excess should be avoided. For this purpose neutralize with 10% $NH_4OH$ until a permanent precipitate forms and add sufficient $NaH_2PO_4$ soln to precipitate all Mg present. Dissolve the precipitate by slowly adding 10% HCl dropwise. Use as little HCl as possible to obtain complete soln. The next step requires considerable care and patience to give accurate results. $MgHPO_4$ begins to precipitate at a pH of 6.7–6.8. This is the critical point. Heat the soln to gentle boiling and add dilute $NH_4OH$ (**f**) at the rate of 4 drops a minute while maintaining a gentle boil until a crystalline precipitate commences to form. The first precipitate must be crystalline not gelatinous. If the first precipitate is gelatinous it is necessary to redissolve it with a little HCl and start the precipitation again more slowly. Stirring assists crystallization but the sides of the beaker should not be scratched. After the crystals have formed in considerable numbers the precipitation may be hastened. This treatment gives crystalline $MgHPO_4$. Continue the addition of the dilute $NH_4OH$ until the soln is slightly ammoniacal. Allow the mixture to cool slightly, then add $\frac{1}{3}$ the volume of $NH_4OH$ slowly, and with constant stirring. Let stand until the precipitate has been converted into $MgNH_4PO_4$, preferably overnight. Filter and wash carefully with dilute $NH_4OH$ (**f**), until all chlorides have been removed. Dry and ignite slowly until all the C is consumed. Cover and ignite intensely. Weigh the white $Mg_2P_2O_7$ and report as MgO. $Mg_2P_2O_7 \times 0.3621 = MgO$. Ignition of dark colored residues with a drop of 20% $NH_4NO_3$ will often improve the color. If the nitrate is added, care must be taken to avoid spattering.

### 23 ALCOHOL PRECIPITATE[2]—TENTATIVE

To 100 cc of soln, prepared as directed under **1** (**b**$_1$) or **1** (**c**$_1$), in a beaker, add 4–8 g of sucrose (1 or 2 lumps of cube sugar) if sugar is not already present, and evaporate to a volume of 20–25 cc. If water-insoluble matter separates during evaporation add more sugar. Cool to room temp. and add slowly and with constant stirring 200 cc of 95% alcohol. Allow to stand at least 1 hour, filter on a 15 cm qualitative paper, and wash the precipitate with 95% alcohol. Wash the precipitate back into the original beaker with hot $H_2O$, rinsing the filter paper thoroly. Evaporate the soln to about 20 cc and add 5 cc of HCl (1+2.5). If water-insoluble matter separates, stir well and, if necessary, warm slightly to dissolve. Again precipitate with 200 cc of 95% alcohol, allow to stand 1 hour, and filter thru paper. Wash the precipitate and paper thoroly with 95% alcohol to remove all HCl. Rinse the precipitate from the filter paper with hot $H_2O$ into a Pt dish, evaporate to dryness on a steam bath, dry to constant weight in a water oven, and weigh; then ignite and weigh again. The loss in weight is the alcohol precipitate.

As the precipitate in many samples is colorless and almost invisible, care must be exercised that none is lost in the dissolving and transferring operations. If the quantity of the alcohol precipitate, as indicated by its volume in the first precipitation, is not excessive, the second filtration may be made thru a Gooch crucible containing a thin asbestos mat. If the alcohol precipitate is very pure and small in quantity it may not be visible at first. In this case, add a small amount of an electrolyte, like NaCl, which will flocculate the alcohol precipitate and render it visible.

### 24 PECTIC ACID[2] (DI-GALACTFURONIC ACID)—TENTATIVE

Transfer a 200 cc aliquot of soln prepared as directed under **1** **($b_1$)** or **1** **($c_1$)** to a beaker, add 8-12 g of sucrose (2 or 3 lumps of cube sugar) if the soln does not already contain sugar, and evaporate to about 25 cc. If organic acids are to be determined in the filtrate from the pectin, cool, add 3 cc of *N* $H_2SO_4$, and immediately add with constant stirring 200 cc of 95% alcohol, allow the precipitate formed to settle, filter on a 15 cm qualitative paper and wash with 95% alcohol. If organic acids are not to be determined, the addition of the $H_2SO_4$ should be omitted. Transfer the precipitate to the original beaker with hot $H_2O$, evaporate to about 40 cc, and cool to 25° or below. If water-insoluble matter separates during evaporation, stir vigorously and, if necessary, add a few drops of HCl (1+2.5) and warm; then cool again. Add 2–5 cc of 10% NaOH soln (the volume of the precipitate will indicate approximately the quantity to use) diluted with sufficient $H_2O$ to make a total volume of 50 cc. Allow to stand 15 min., add 40 cc of $H_2O$ and 10 cc of HCl (1+2.5), and boil for 5 min. Filter, and wash the precipitate of pectic acid with hot $H_2O$. This filtration should be rapid and the filtrate clear. If the filtrate is cloudy or of a colloidal nature, reject the determination. Colloidal filtrates are due to insufficient alkali or to saponification at too high a temp., or both. In such cases, repeat the determination, using more alkali and keeping the temp. low. Wash the precipitate of pectic acid back into the beaker; adjust to a volume of 40 cc; cool to below 25°; and repeat the saponification with dilute NaOH soln, the precipitation with dilute HCl, and the boiling as above described. Again filter and wash the precipitate of pectic acid with hot $H_2O$, but only to the point when a test of the filtrate shows a negligible quantity of acid. Not more than 500 cc of total filtrate should be necessary. Wash the pectic acid into a Pt dish and dry on a steam bath and finally in a water oven to constant weight. Weigh, ignite, and weigh again. The loss in weight is pectic acid.

### 25 PROTEIN—OFFICIAL

Proceed as directed under **II, 19, 22** or **24,** using 5 g of jelly or other fruit product containing a large quantity of sugar, or 10 g of juice or fresh fruit, and a larger quantity of the $H_2SO_4$ if necessary for complete digestion. Multiply the percentage of N by 6.25 to obtain the percentage of protein.

### 26 TOTAL ACIDITY—OFFICIAL

Dilute 10 g of juice, prepared as directed under **1** **(a)**, or 25 cc of soln, prepared as directed under either **1** **($b_1$)** or **1** **($c_1$**, with recently boiled $H_2O$ to about 250 cc, or less if the sample is not highly colored. Titrate with 0.1 *N* alkali, using phenolphthalein indicator. In the case of highly colored products, employ azolitmin soln or phenolphthalein powder, **XVII, 44,** on a spot plate instead of phenolphthalein soln. Report the result as cc of 0.1 *N* alkali per 100 g or 100 cc of the original material.

### 27 VOLATILE ACIDS—OFFICIAL

Dissolve 10 g of the sample; dilute to 25 cc; and distil in a current of steam, as directed under **XVII, 46.** Each cc of 0.1 *N* alkali = 0.0060 g of acetic acid.

## TARTARIC ACID[6]—TENTATIVE

28

### REAGENTS

(a) *Di-ammonium citrate soln.*—50 g to the liter. Neutralize 43.5 g of crystalline citric acid with $NH_4OH$, evaporate to dryness on the water bath, and make up to 1 liter.

(b) *Ammonium l-tartrate soln.*—Dilute 20 g, rigorously free from d-tartrate, to 1 liter and add 5 or 6 cc of formalin to the soln as a preservative.

(c) *Calcium acetate soln.*—Dissove 16 g of $CaCO_3$ in 120 cc of glacial acetic acid diluted with sufficient $H_2O$, filter, and make up to 1 liter.

(d) *Hydrochloric acid soln.*—Dilute 40 g of the concentrated acid to 1 liter.

(e) *Calcium carbonate soln.*—Dissolve 5 g in 20 g of acetic acid, add 100 g of Na-acetate, and make up to 1 liter.

(f) *Potassium permanganate soln.*—6.9745 g per liter. Standardize this soln against a soln of pure tartaric acid of known titer. 1 cc of the $KMnO_4$ should correspond to nearly 0.005 g of tartaric acid.

(g) *Oxalic acid soln.*—Dilute 13.8793 g to 1 liter and titrate against the $KMnO_4$ soln.

Carry out the standardization of the $KMnO_4$ against tartaric acid just as prescribed for the determinations, namely, dilute the tartaric acid soln to 50–60 cc with a soln of $H_2SO_4$, 10% by volume. Heat nearly to boiling in a casserole and run the $KMnO_4$ in slowly until it ceases to be decolorized on several minutes' standing.

Add the standard oxalic until the $KMnO_4$ color has disappeared, or, if preferred, add a slight excess of oxalic soln and obtain the end point with $KMnO_4$. (It is to be noted that the final end point with tartaric acid is not obtained so easily as with oxalic acid, hence the oxalic end point is preferred. However, the tartaric acid must be completely oxidized, as the $KMnO_4$ fades rather slowly towards the end of the titration.

29

### DETERMINATION

To determine tartaric acid, whether in the presence of troublesome metals or not, bring the soln to a volume of 150 cc, add 10–15 cc of reagent (a), 25 cc of (b), and 20 cc of (c); stir well, and allow to stand, with occasional stirring, for at least 4 hours; if interfering metals are present, allow to stand 12 hours. Filter the precipitate on a Gooch crucible with a thin mat and wash six times with 10 cc portions of $H_2O$.

Dissolve the precipitate, after removal from the Gooch with the mat, with 20 cc of (d) and wash the Gooch thoroly. Then adjust the volume of the soln to 150 cc, add 40–50 cc of (e), and bring the temp. to 80° on the water bath. Cool the soln, stir, and allow to stand several hours. Refilter the precipitate on a Gooch and wash as before.

Remove the soln and the asbestos to a casserole, adding 50–60 cc of warm 10% $H_2SO_4$ by volume and pouring thru the Gooch in order to wash out any traces of the precipitate adhering to it.

Bring the soln in the casserole near the boiling point and add the standard permanganate (f) slowly so long as decolorized. When the color remains several min., run in a slight excess of standard oxalic acid soln (g) and add $KMnO_4$ till a slight pink tinge is apparent.

The weight of tartaric acid represented by the $KMnO_4$ consumed in oxidizing the tartaric acid divided by two gives the weight of tartaric acid in the sample under examination.

## TARTARIC ACID[6] (AS ACID POTASSIUM TARTRATE)—TENTATIVE

### 30 PREPARATION OF SAMPLE

With products containing large quantities of sugars, such as jams and jellies, use 200 cc of a sample soln prepared as directed under **1** (**b**) or **1** (**c**). In the case of fruit juices or products of similar nature use a portion of the material containing not more than 200 mg of acid calculated as tartaric acid.

Determine the acidity of the portion taken in terms of normal acid (titratable acidity), adjust to a volume of approximately 35 cc, either by evaporation or by the addition of $H_2O$, add 3 cc of $N$ $H_2SO_4$, and heat to 50°.

Designate the acidity of the soln as "A" (titratable acidity +3). Pour the contents of the beaker into a 250 cc volumetric flask, rinse the beaker with about 15 cc or warm $H_2O$ and finally with 95% alcohol, make to mark with 95% alcohol, shake, allow to stand 15 min., and filter thru a thin layer of absorbent cotton. Filtration is usually fast, but if it is necessary to renew the cotton, squeeze the liquid onto the new filter. During filtration cover the funnel with a watch-glass to retard evaporation of alcohol. Transfer 200 cc of the clear filtrate to a 16 oz. centrifuge bottle. The filtrate from the first precipitation of pectic acid when $H_2SO_4$ has been added, **24**, is suitable for the determination of organic acids.

In the case of products containing alcohol, saponification is necessary. For this purpose adjust the volume of the portion taken to about 35 cc, add 3 cc of $N$ KOH in excess of that required for neutralization, heat to boiling, and allow to stand overnight. Add $N$ $H_2SO_4$ equal to the total quantity of $N$ alkali added and 3 cc in excess. Transfer to a 250 cc volumetric flask as described previously. "A" in this case = the number of cc of $N$ $H_2SO_4$ used + the titratable acidity of the material.

### 31 REAGENTS

*Lead acetate soln.*—Approximately 1.5 $N$. Dissolve 70 g of Pb-acetate in $H_2O$, add 1 cc of glacial acetic acid, and dilute to 250 cc.

### 32 DETERMINATION

To the material in the centrifuge bottle add a quantity of Pb-acetate soln equal to 0.8 "A," shake vigorously for 2 min., and centrifugalize at about 900 r.p.m. for 15 min. Carefully decant the supernatant liquid from the precipitated Pb salts and if sediment lifts, repeat the centrifugalizing, increasing the speed and time. Allow the salts to drain thoroly by inverting the bottle for several min. To the material in the centrifuge bottle add 150 cc of 80% alcohol, shake vigorously, and again centrifugalize, decant, and drain. Transfer the salts to a 400 cc beaker with about 150 cc of $H_2O$, warm and pass in a rapid stream of $H_2S$ until the soln is cool, *stirring frequently*. Transfer to a 250 cc volumetric flask, make to mark with $H_2O$, and filter thru a folded filter. Transfer 225 cc of the clear filtrate to a 400 cc beaker, add several small glass beads to facilitate boiling, and evaporate to 20 cc over a small flame. Neutralize with $N$ KOH, using phenolphthalein as indicator, and add 3 drops of the alkali in excess. Add 2 cc of glacial acetic acid and 80 cc of 95% alcohol slowly and with constant stirring. Chill in an ice bath, stir vigorously for 2 min., and place in the refrigerator overnight. Carefully decant the supernatant liquid onto a thin pad of asbestos in a Gooch crucible with a removable bottom, leaving about 25 cc in the beaker. To the contents of the beaker add about 0.3 g of dry purified asbestos, mix thoroly, and transfer to the crucible with three portions of about 15 cc each of ice-cold 80% alcohol and finally wash the contents of the crucible with three portions of 15 cc each of the ice-cold alcohol, sucking the crucible dry each time. Transfer the pad and precipitate to a 250 cc beaker with about 100 cc of hot $H_2O$, bring almost to boiling, and titrate with 0.1 *N* alkali, using phenolphthalein as indicator. 1 cc of 0.1 *N* alkali = 0.015 g of tartaric acid.

## CITRIC ACID

### 33 PREPARATION OF SAMPLE

Proceed as directed under **30**.

### 34 REAGENTS

(a) *Potassium bromide soln.*—Dissolve 15 g of KBr in 40 cc of $H_2O$.

(b) *Potassium permanganate soln.*—Dissolve 5 g of $KMnO_4$ in $H_2O$ and dilute to 100 cc.

(c) *Ferrous sulfate soln.*—Dissolve 40 g of $FeSO_4.7H_2O$ in 100 cc of $H_2O$ containing 1 cc of $H_2SO_4$.

(d) *Lead acetate soln.*—Approximately 1.5 *N*. Dissolve 70 g of Pb-acetate in $H_2O$, add 1 cc of glacial acetic acid, and dilute to 250 cc.

### 35 DETERMINATION

To the material in the centrifuge bottle, add a quantity of the Pb-acetate soln equal to 0.8 "A" (cf. **30**), shake vigorously for 2 min., and centrifugalize at about 900 r. p. m. for 15 min. Carefully decant the supernatant liquid from the precipitated Pb salts. If sediment lifts, repeat the centrifugalizing, increasing the speed and time. Allow the mixture to drain thoroly by inverting the bottle for several min. To the material in the centrifuge bottle add 150 cc of 80% alcohol, shake vigorously, and again centrifugalize, decant, and drain. Transfer the salts to a 400 cc beaker with about 150 cc of $H_2O$; warm, and pass in a rapid stream of $H_2S$ until the soln is cool, *stirring frequently.* Transfer to a 250 cc volumetric flask, make to mark with $H_2O$, and filter thru a folded filter. Transfer 225 cc of the filtrate to a 500 cc Erlenmeyer flask, add several small glass beads to facilitate boiling, evaporate to about 75 cc, cool, and add 10 cc of $H_2SO_4$ (1+1) and 5 cc of the KBr soln. Heat the mixture to 48–50° and maintain this temp. for 5 min. To the warm soln add immediately 50 cc of the $KMnO_4$ soln; shake vigorously in the stoppered flask for about 1 min., releasing the pressure frequently; and allow to stand 4 min. Do not permit the temp. to exceed 55°. (During this time there should be a heavy deposit of $MnO_2$; if necessary, add more $KMnO_4$ to assure an excess of the oxidizing agent. If at any time during the oxidation the precipitated $MnO_2$ disappears, discard the determination and repeat, using more $KMnO_4$.) Remove $MnO_2$ with the $FeSO_4$ soln (20 cc is generally sufficient), cool, shake vigorously, and place in the refrigerator overnight. Filter by decantation onto a thin tightly-tamped pad of asbestos in a Gooch crucible. It is important that filtration be completed as quickly as possible. Note volume of filtrate and use this filtrate to transfer the precipitate to the crucible. Wash the contents of the crucible at once with 50 cc of ice-cold $H_2O$. Dry the pentabromacetone by aspirating with dry air or in a sulfuric acid vacuum desiccator and weigh. Remove the pentabromacetone by treating the contents of the crucible with three portions of 20 cc each of alcohol and three portions of 20 cc each of ether. Again dry and weigh. The difference in the two weights represents the weight of pentabromacetone. Calculate the citric acid by the following formula:

$X = 1.05\ (0.424P + 0.017S)$, in which
$X$ = mg of citric acid in aliquot,
$P$ = weight of pentabromacetone in mg, and
$S$ = volume of filtrate (cc).

36

For drying the pentabromacetone by aspiration, use the following apparatus (Fig. 26).

*A*, Gooch crucible (28 mm diam.) loosely packed with cotton;
*B*, Gooch crucible (35 mm diam.) for pentabromacetone; and
*C*, Suction flask (about 500 cc capacity).

Dry the air by passing it thru $H_2SO_4$ and soda-lime, and finally filter thru cotton. Cool the air entering the drying train by passing it thru a spiral condenser cooled with $H_2O$.

Allow the crucible *B*, containing the pentabromacetone to remain under suction for about 1 min. to remove surface moisture before placing in the apparatus. If the air does not pass thru freely, place the crucible in a desiccator for a short time. Maintain a slow uniform flow of air by just "cracking the suction." Dry until the loss in weight does not exceed a few tenths of a mg, making the first weighing after 20 min.

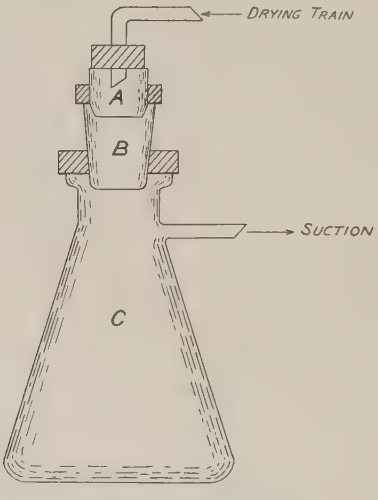

FIG. 26.—APPARATUS FOR DRYING PENTABROMACETONE BY ASPIRATION.

### 37 FREE MINERAL ACIDS—TENTATIVE

Proceed as directed under **XXXIII, 72–74.**

## SUCROSE

### 38 *By Polarization—Official*

Determine by polarizing before and after inversion, as directed under **XXXIV, 22, 23** or **27.**

### 39 *By Reducing Sugars before and after Inversion—Official*

Proceed as directed under **XXXIV, 28.**

### 40 REDUCING SUGARS—OFFICIAL

Proceed as directed under **XXXIV, 38,** and express the results as invert sugar.

### 41 COMMERCIAL GLUCOSE—OFFICIAL

Proceed as directed under **XXXIV, 30.**

### 42 DEXTRIN—TENTATIVE

Dissolve 10 g of the sample in a 100 cc flask and add 20 mg of KF and then about ¼ of a cake of compressed yeast. Allow the fermentation to proceed below 25° for 2–3 hours to prevent excessive foaming and then incubate at 27–30° for 5 days. At the end of that time, clarify with basic Pb-acetate soln and alumina cream; make up to 100 cc, filter, and polarize in a 200 mm tube. A pure fruit jelly will show a dextro or levo rotation of not more than a few tenths of a degree. If a polariscope having the Ventzke scale is used and a 10% soln is polarized in a 200 mm tube, the number of degrees read on the sugar scale of the instrument ×0.8755 = the percentage of dextrin; or the following formula may be used:

$$\text{Percentage of dextrin} = \frac{C \times 100}{198 \times L \times W}, \text{ in which}$$

$C$ = degrees of circular rotation;
$L$ = length of tube in decimeters; and
$W$ = weight of sample in 1 cc.

## STARCH

### 43 *Qualitative Test—Official*

Dilute a portion of the sample with $H_2O$, heat nearly to boiling, add several cc of $H_2SO_4$ (1+9), and then add $KMnO_4$ soln until all color is destroyed. Cool, and test with I soln [**XXXIII, 20** (j)]. The presence of starch is not necessarily an indication of its addition as an adulterant. It is usually present in small quantity in the apple, and occasionally in other fruits, and unless it is found in the fruit product in considerable quantity its presence may be due to these natural sources.

## GELATIN[8]

### 44 *Qualitative Test—Tentative*

The presence of gelatin in jellies and jams is shown by the increased content of N. Precipitate a concentrated soln of jelly or jam with 10 volumes of absolute alcohol and determine N in the dried precipitate as directed under **II, 19, 22** or **24.**

## AGAR AGAR

### 45 *Detection by Microscopic Examination[9]—Tentative*

Heat the jelly with $H_2SO_4$ (1+18), add a crystal of $KMnO_4$, and allow to settle. If agar agar is present, the sediment will be rich in diatoms, which can be detected by the use of the microscope. The diatoms adhere to the glass and are best obtained by pouring out the liquid, washing the glass with 2 or 3 drops of alcohol, and transferring the alcohol to a microscopic slide by means of a glass rod.

### 46 *Detection by Precipitation[9]—Tentative*

Cover 30 g of the jam or jelly with 270 cc of hot $H_2O$, stir until thoroly disintegrated, and boil for 3 min. Filter immediately, while still boiling hot, thru a rapid qualitative filter paper. In the presence of agar agar a precipitate will form upon standing not longer than 24 hours. Filter, wash with cold $H_2O$, and dissolve from the paper by means of a very small quantity of boiling $H_2O$. Upon chilling this hot water soln a firm jelly that can be examined by the touch will be formed. This method will detect 0.2% of agar agar with certainty if the proportions of jam or jelly and $H_2O$ are strictly observed.

## 47 ADDED WATER IN GRAPE JUICE[11]—TENTATIVE

(Applicable to white juices only)

Transfer about 50 cc of the filtered juice to a 2-oz. tincture bottle containing 10 glass rods, 15 mm long and 5 mm in diameter, and approximately 1 g of finely powdered cream of tartar. Cork the bottle tightly, place it neck downward in a pint fruit jar, fill the jar with $H_2O$ at 25°, and hold at this temp. for 30 min., shaking occasionally. (By placing the jar in a bucket of $H_2O$, the temp. of 25° can be easily controlled.) At the end of this time seal the fruit jar, wrap it in three sheets of heavy wrapping paper, making three separate wrappings, place in a mechanical shaker, and shake for 1 hour. After the shaking has been accomplished, ascertain the temp. ($t°$) of the $H_2O$ in the jar. Filter the juice immediately and titrate 10 cc with 0.1 $N$ NaOH, using phenolphthalein indicator. Titrate in the same manner 10 cc of the original juice. The two titrations should be made side by side in order to obtain the same shade of pink with the greatest possible accuracy. Calculate the percentage of added sugar soln ($H_2O$) from the following formula:

$$W = \frac{0.0188\,(b-a) - 0.095 - 0.025\xcancel{\left(\frac{2}{t^\circ - 25}\right)}}{0.006} \quad \frac{t^\circ - 25}{2}, \text{ in which}$$

$W$ = percentage of volume of added $H_2O$ (20% sugar soln);
$b$ = acidity of treated juice, cc 0.1 $N$ NaOH per 100 cc;
$a$ = acidity of original juice, cc 0.1 $N$ NaOH per 100 cc; and
$t°$ = temp. after shaking.

Pure factory juices examined by this method show a small quantity of added $H_2O$ varying from 1 to 3%.

## 48 METALS—TENTATIVE

Proceed as directed under **XXIX.**

## 49 PRESERVATIVES—OFFICIAL

Proceed as directed under **XXXII.**

## 50 COLORING MATTERS—TENTATIVE

Proceed as directed under **XXI.**

## 51 SWEETENING SUBSTITUTES

Proceed as directed under **XXXII, 13, 14, 38, 39.**

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# XXVII. GRAIN AND STOCK FEEDS

## GENERAL METHODS

### 1 PREPARATION OF SAMPLE—OFFICIAL

Grind the sample to pass thru a sieve having circular openings 1/25 inch (1 mm) in diameter and mix thoroly. If the sample cannot be ground, reduce it to as fine a condition as possible.

### MOISTURE[1]

### 2 *I. Drying with Heat—Official*

Dry to constant weight at the temp. of boiling $H_2O$ under a pressure not to exceed 100 mm of Hg (approximately 5 hours), a quantity of the substance representing about 2 g of dry material; or dry in a current of H at the same temp. but at atmospheric pressure. If the substance is contained in a glass vessel, the latter should not come in contact with the boiling $H_2O$. Report the loss in weight as moisture.

### *II. By Distillation with Toluene—Official*

### 3 APPARATUS

A 250 cc distilling flask of Pyrex or other resistant glass connected by means of a "distilling tube receiver" to a 20-inch, sealed-in, straight-tube Liebig condenser with delivery tube not over 5/16 inch in the manner shown in Fig. 27. The distilling tube receiver is of the dimensions shown and is made by attaching a proper side tube to the calibrated section of a 5 cc Mohr pipet and sealing the outlet. The tube is calibrated in lengths of a cc by distilling known quantities of $H_2O$ into the graduated column, and the column of $H_2O$ may be read to hundredths with reasonable accuracy. Clean the tube and condenser with $Cr_2O_3$-$H_2SO_4$ mixture, rinse thoroly with $H_2O$, then with alcohol, and dry in an oven to prevent an undue quantity of $H_2O$ adhering to the inner surfaces during the determination.

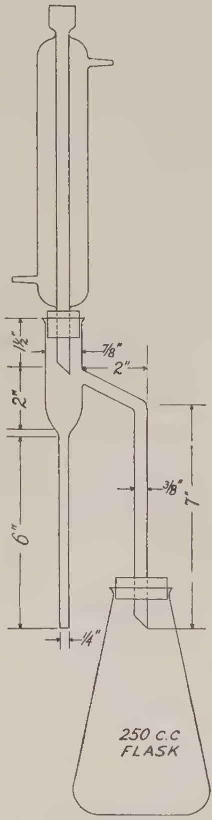

FIG. 27.—APPARATUS USED IN METHOD II FOR THE DETERMINATION OF MOISTURE.

### 4 DETERMINATION

If the sample is likely to bump, add enough dry sand to cover the bottom of the flask. Add sufficient toluene to cover the sample completely (about 75 cc). Weigh and introduce into the toluene sufficient sample to give 2-5 cc of $H_2O$ and connect the apparatus as shown in Fig. 27. Fill the receiving tube with toluene, pouring it thru the top of the condenser. Bring to a boil and distil slowly about 2 drops per second, until most of the $H_2O$ has passed over: then increase the rate of distillation to about 4 drops per second. When all the $H_2O$ is apparently over, wash down the condenser by pouring toluene in at the top, continuing the distillation a short time to ascertain whether any more $H_2O$ will distil over; if it does, repeat the washing down process.

If any $H_2O$ remains in the condenser, remove it by brushing down with a tube brush attached to a Cu wire and saturated with toluene, washing down the condenser at the same time. The entire process is usually completed within an hour. Allow the receiving tube to come to room temp. If any drops adhere to the sides of the tube, force them down by means of a rubber band wrapped around a Cu wire. Read the volume of $H_2O$ and calculate to percentage.

### *III. Drying without Heat over Sulfuric Acid*[2]*—Official*

### 5 REAGENT

*Sulfuric acid.*—Boil $H_2SO_4$ in a large Kjeldahl flask for 4 hours, close the mouth of the flask with a stopper carrying a $CaCl_2$ tube, and cool.

### 6 DETERMINATION

Weigh a suitable quantity of the sample, usually 2–5 g, into a metal dish 5–10 cm in diameter and provided with a tightly fitted cover. If subsequent fat determinations are to be made, fat extraction cones may be used. Substances that dry down to horn-like material should be mixed with fat-free cotton or other suitable material. Place 200 cc of the fresh $H_2SO_4$ in a strong, tight vacuum desiccator. Then place the dish, uncovered, in the desiccator and exhaust by means of a vacuum pump to a pressure of not more than 10 mm of Hg.

If a pump is not available, place 10 cc of ether in a small beaker in the desiccator and exhaust with a water filter pump. Between the pump and the desiccator interpose an empty bottle next to the desiccator and a bottle of $H_2O$ next to the pump. Draw the air from the desiccator thru the $H_2O$ and turn the desiccator stopcock the instant the $H_2O$ begins to rise in the tube leading from the empty bottle.

Gently rotate the desiccator 4 or 5 times during the first 12 hours. At the end of 24 hours open the desiccator, causing the incoming air to bubble thru $H_2SO_4$, place the cover on the dish, and make the first weighing. After weighing place the sample in a desiccator containing fresh $H_2SO_4$ and exhaust as before. Rotate the desiccator several times during the interval and weigh again after a suitable period of drying. Repeat this process until the weight is constant.

### 7 *IV. Electric Air-Oven Method*[3]*—Official, first action*

Regulate an electric air oven to 135°, ±2°. Using low, covered Al dishes, weigh approximately 2 g of the sample into each dish and shake until the contents are evenly distributed. With the covers removed, place the dishes and covers in the oven as quickly as possible and dry the samples for 2 hours. After placing the covers on the dishes transfer them to a desiccator to cool. Weigh and calculate the loss in weight as moisture.

### 8 ASH—OFFICIAL

Weigh a quantity of the substance representing about 2 g of dry material and burn at a low heat, not exceeding dull redness, until free from C. If a C-free ash cannot be obtained in this manner, exhaust the charred mass with hot $H_2O$, collect the insoluble residue on an ashless filter, and burn the filter and contents to a white or nearly white ash. Add the filtrate, evaporate to dryness, and heat at dull redness until the ash is white or grayish white. Cool in a desiccator and weigh.

### 9 SALT[4] (QUALITATIVE)—OFFICIAL, FIRST ACTION

Transfer 2 cc of a 5% soln of $AgNO_3$ to a small test tube of 1 cm internal diameter. Carefully add to this liquid an equal volume of the feed, which previously has been ground to pass an mm sieve, so that most of the sample floats or remains above the liquid. Gradually incline the tube so that the liquid is absorbed. White patches of AgCl appear wherever the minutest crystal of salt comes in contact with the soln. These patches may easily be observed with a lens or even with the naked eye.

### 10 CRUDE PROTEIN—OFFICIAL

Determine N as directed under **II, 19, 22,** or **24,** and multiply the result by 6.25.

## ALBUMINOID NITROGEN—OFFICIAL

### 11 REAGENT

*Cupric hydroxide.*—Dissolve 100 g of $CuSO_4.5H_2O$ in 5 liters of $H_2O$; add 2.5 cc of glycerol, and then add 10% NaOH soln until the liquid is slightly alkaline; filter; rub the precipitate in a mortar with $H_2O$ containing 5 cc of glycerol per liter; and wash by decantation or filtration until the washings are no longer alkaline. Again rub the precipitate in a mortar with $H_2O$ containing 10% of glycerol, thus preparing a uniform gelatinous mass that can be measured with a pipet. Determine approximately the quantity of $Cu(OH)_2$ in 5 cc by diluting to 50 cc with $H_2O$, filtering, washing, igniting, and weighing as CuO.

### 12 DETERMINATION

Place 0.7 g of the sample in a beaker, add 100 cc of $H_2O$, and heat to boiling; or, in case of substances rich in starch, heat on a steam bath for 10 min.; add a quantity of the $Cu(OH)_2$ reagent that contains about 0.5 g of the hydroxide; stir thoroly; filter when cold; wash with cold $H_2O$; and, without removing the precipitate from the filter, determine the N as directed under **II, 19, 22,** or **24,** adding sufficient $K_2S$ or $Na_2S$ soln [**II, 17 (h)**] to precipitate all the Cu and Hg. The filter paper used must be practically free from N. If the material (such as seeds, seed residue, or oil cake) is rich in alkaline phosphates, add 1–2 cc of a 10% soln of $NH_3$-free soda alum to decompose the alkaline phosphates, then the $Cu(OH)_2$ reagent, and mix well by stirring. If this is not done, Cu-phosphate and free alkali may be formed and the protein-copper precipitate partially dissolved in the alkaline liquid.

### 13 AMIDO NITROGEN—OFFICIAL

Subtract the percentage of albuminoid N from the percentage of total N to obtain the amido N.

## CRUDE FAT OR ETHER EXTRACT

### *I. Direct Method—Official*

### 14 REAGENT

*Anhydrous ether.*—Wash commercial ether with 2 or 3 successive portions of $H_2O$, add solid NaOH or KOH, and let stand until most of the $H_2O$ has been abstracted from the ether. Decant into a dry bottle, add small pieces of carefully cleaned metallic Na, and let stand until there is no further evolution of H gas. Keep the ether, thus dehydrated, over metallic Na in loosely stoppered bottles.

### 15 DETERMINATION

Large quantities of soluble carbohydrates may interfere with the complete extraction of the fat. In such cases extract with $H_2O$ before proceeding with the determination. Extract about 2 g of the sample, dried as directed under **2, 6,** or **7,** with the anhydrous ether for 16 hours. Dry the extract at the temp. of boiling $H_2O$ for 30 min., cool in a desiccator, and weight; continue, at 30 min. intervals, this alternate drying and weighing until the weight is constant. For most feeds a period of 1–1.5 hours is required.

### 16 *II. Indirect Method—Official*

Determine moisture, as directed under **2**, **6**, or **7**; then extract the dried substance for 16 hours as directed under **15**, and dry again. Report the loss in weight as ether extract.

## CRUDE FIBER[5]—OFFICIAL

### 17 REAGENTS

(**a**) *Sulfuric acid soln.*—Contains 1.25 g of $H_2SO_4$ per 100 cc of $H_2O$.

(**b**) *Sodium hydroxide soln.*—Contains 1.25 g of NaOH per 100 cc, free, or nearly so, from $Na_2CO_3$.

The strength of these solns must be accurately checked by titration.

(**c**) *Asbestos.*—Digest on a steam bath or at an equivalent temp. for at least 8 hours with an approximately 5% NaOH soln and thoroly wash with hot $H_2O$; then digest in a similar manner for 8 hours with HCl (1+3) and again wash thoroly with hot $H_2O$; dry; and ignite at bright red heat.

### 18 APPARATUS

(**a**) *Condenser.*—Use a condenser that will maintain a constant volume of soln thruout the process of digestion.

(**b**) *Digestion flasks.*—Use digestion flasks of such size and shape that the soln will be not less than 1 inch nor more than 1½ inches in depth. A 700–750 cc Erlenmeyer flask is recommended.

(**c**) *Filtering cloth.*—Use filtering cloth of such character that no appreciable solid matter passes thru when filtering is rapid. Butcher's linen or dress linen with about 45 threads to the inch or No. 40 filtering cloth made by the National Filter Cloth and Weaving Company, or its equivalent, may be used.

### 19 DETERMINATION

Extract 2 g of the dry material with ordinary ether, or use the residue from the ether extract determination (**15** or **16**), and transfer the residue together with about 0.5 g of asbestos, to the digestion flash. (If the residue from the ether extract is used and the proper quantity of asbestos has already been added, further addition is unnecessary.) Add 200 cc of the boiling dilute $H_2SO_4$, immediately connect with the condenser, and heat. It is essential that the contents of the flask come to boiling within 1 min. and that the boiling continue briskly for exactly 30 min. Rotate the flask about every 5 min. in order to mix the charge thoroly. Take care to keep the material from remaining on the sides of the flask out of contact with the soln. (A blast of air conducted into the flask will serve to reduce frothing of the liquid.) At the expiration of 30 min. remove the flask, immediately filter thru linen in a fluted funnel, and wash with boiling $H_2O$ until the washings are no longer acid. Bring a quantity of the dilute NaOH soln to boiling and keep at this temp. under a reflux condenser until used. Wash the charge and asbestos back into the flask with 200 cc of the boiling dilute NaOH soln, using a wash bottle marked to deliver 200 cc. (The boiling NaOH soln is conveniently transferred to the 200 cc wash bottle by means of a bent tube thru which the liquid is forced by blowing into a tube connected with the top of the reflux condenser attached to the NaOH flask.) Then connect the flask with the reflux condenser and boil for exactly 30 min. The boiling with the alkali should be so timed that the contents of the different flasks will reach the boiling point approximately 3 min. apart, which permits sufficient time for filtration. At the expiration of 30 min. remove the flask and immediately filter thru a Gooch prepared with an asbestos mat, thru an alundum crucible, or thru the filtering cloth in a fluted funnel. If the filtering cloth is used, thoroly wash the residue with boiling $H_2O$ and then transfer it to a Gooch crucible prepared with a thin but close layer of ignited asbestos. After thoro washing with boiling $H_2O$, wash with about 15 cc of 95% alcohol. Dry the crucible and contents at 110° to constant weight. Cool in an efficient desiccator and weigh. Incinerate the contents of the crucible in an electric muffle or over a Meker burner at a dull red heat until the carbonaceous matter has been consumed—20 min. is usually sufficient. Cool in a desiccator and weigh. Report the loss in weight as crude fiber.

## SUGARS[6]

### 20 PREPARATION OF SOLUTION—OFFICIAL, FIRST ACTION

Place 10 g of the material in a 250 cc volumetric flask. If the substance has an acid reaction, add 1–3 g of $CaCO_3$ to neutralize the acidity. Add 125 cc of 50% alcohol by volume; mix thoroly; and boil on a steam bath for 1 hour, using a small funnel in the neck of the flask to condense the vapor. Cool, and allow the mixture to stand several hours, preferably overnight. Make up to volume with neutral 95% alcohol, mix thoroly, and allow to settle. Pipet 200 cc of the supernatant soln into a beaker and evaporate on a steam bath to a volume of 20–30 cc. Do not evaporate to dryness. A little alcohol in the residue does no harm. Transfer to a 100 cc volumetric flask and rinse the beaker thoroly with $H_2O$, adding the rinsings to the contents of the flask. Add enough saturated neutral Pb-acetate soln to produce a flocculent precipitate, shake thoroly, and allow to stand 15 min. Dilute to the mark with $H_2O$, mix thoroly, and filter thru a dry filter. Add sufficient anhydrous $Na_2CO_3$ or K-oxalate to the filtrate to precipitate all the Pb, again filter thru a dry paper, and test the filtrate with a little anhydrous $Na_2CO_3$ or K-oxalate to make sure that all the Pb has been removed.

### 21 REDUCING SUGARS—OFFICIAL, FIRST ACTION

Proceed as directed under **XXXIV, 38** or **61**, using 25 cc of the soln (representing 2 g of the sample), prepared as directed under **20.** Express the results as dextrose or invert sugar.

### 22 SUCROSE—OFFICIAL, FIRST ACTION

Introduce 50 cc of the soln prepared as directed under **20** into a 100 cc volumetric flask; add a piece of litmus paper; neutralize with HCl; add 5 cc of HCl; and allow the inversion to proceed at room temp. as directed under **XXXIV, 23 (c)**. When inversion is complete, transfer the soln to a beaker; neutralize with $Na_2CO_3$; return the soln to the 100 cc flask; dilute to the mark with $H_2O$; filter if necessary; and determine reducing sugars in 50 cc of the soln (representing 2 g of the sample) as directed under **21.** Calculate the results as invert sugar. Subtract the percentage of reducing sugars before inversion from the percentage of total sugar after inversion, both calculated as invert sugar, and multiply the difference by 0.95 to obtain the percentage of sucrose present.

Since the insoluble material of grain or cattle food occupies some space in the flask as originally made up, it is necessary to correct for this volume. Results of a large number of determinations on various materials have shown the average volume of 10 g of material to be 7.5 cc, and therefore to obtain the true quantity of sugars present all results must be multiplied by the factor 0.97.

## STARCH

### 23 *I. Direct Acid Hydrolysis—Official*

(This method is intended only for such materials as raw starch, potatoes, etc., and includes as starch the pentosans and other carbohydrate bodies that undergo hydrolysis and are converted into reducing sugars on boiling with HCl.)

Stir a weighed quantity of the sample, representing 2.5–3 g of the dry material, in a beaker with 50 cc of cold $H_2O$ for an hour. Transfer to a filter and wash with 250 cc of cold $H_2O$. Heat the insoluble residue for 2.5 hours with 200 cc of $H_2O$ and 20 cc of HCl (sp. gr. 1.125) in a flask provided with a reflux condenser. Cool, and nearly neutralize with NaOH. Complete the volume to 250 cc, filter, and determine the dextrose in an aliquot of the filtrate as directed under **XXXIV, 59** or **61.** The weight of the dextrose obtained multiplied by 0.90 gives the weight of starch.[7]

### *II. Diastase Method with Subsequent Acid Hydrolysis—Official*

### 24 REAGENT

Use the reagent described under **26.**

### 25 DETERMINATION

Extract a convenient quantity of the substance (ground to an impalpable powder and representing 4–5 g of the dry material) on a hardened filter with 5 successive portions of 10 cc of ether; wash with 150 cc of alcohol, 10% by volume, and then with a few cc of 95% alcohol. Place the residue in a beaker with 50 cc of $H_2O$, immerse the beaker in boiling $H_2O$, and stir constantly for 15 min., or until all the starch is gelatinized; cool to 55°; add 20 cc of the malt extract; and maintain at this temp. for an hour. Heat again to boiling for a few min., cool to 55°, add 20 cc of the malt extract, and maintain at this temp. for an hour, or until the residue treated with I soln shows no blue color upon microscopical examination. Cool, make up directly to 250 cc, and filter. Place 200 cc of the filtrate in a flask, add 20 cc of HCl (sp. gr. 1.125), connect with a reflux condenser and heat in a boiling water bath for 2.5 hours. Cool, nearly neutralize with 10% NaOH soln, finish the neutralization with $Na_2CO_3$ soln, and dilute to 500 cc. Mix the soln thoroly, pour thru a dry filter, and determine the dextrose in an aliquot as directed under **XXXIV, 59** or **61.** Conduct a blank determination upon the same volume of the malt extract as used with the sample and correct the weight of dextrose accordingly. The weight of the dextrose obtained, multiplied by 0.90, gives the weight of starch.

### *III. In the Presence of Interfering Polysaccharides[8]—Official*

### 26 REAGENT

*Malt extract.*—Use clean, new barley malt of known efficacy and grind only as needed. Grind well, but not so fine that filtration will be greatly retarded. Prepare an infusion of the freshly ground malt just before it is to be used. For every 80 cc of the malt extract required digest 5 g of the ground malt with 100 cc of $H_2O$, at room temp., for 2 hours, or for 20 min. if the mixture can be stirred by an electric mixer. Filter to obtain a clear extract. (It may be necessary to return the first portions of the filtrate to the filter.) Mix the infusion well.

### 27 DETERMINATION

Weigh 2–6 g (charges of 4 g for linseed meal, or 3 g for dried apple pomace, have been found to be satisfactory) of the well-mixed sample, prepared to pass freely thru a sieve not less than 40 mesh to the inch, using the smaller charges in the case of materials containing much gel-forming substance. (The weight of starch in the charge must not exceed 1.5 g.) Transfer to a dry 12.5-15 cm close-textured rapid filtering paper in a glass funnel and extract with 5 successive portions of ether, taking for each portion more than enough to cover the charge and using a cover-glass to retard evaporation. After completing the ether extraction, allow the ether to evaporate and then extract the charge with 300 cc of dilute alcohol. The concentration of the alcohol may be varied somewhat to suit the material under examination. For linseed meal use 35% alcohol (by volume) and for dried apple pomace use 25% alcohol. Follow this with several filterfuls of 95% alcohol and finish the leaching operations with a second ether extraction. Conduct also a control determination, preferably in duplicate, using a filter paper extracted with alcohol and the same quantity of $H_2O$ and malt extract as in the determination. (It is convenient to let the charge stand overnight at this point to allow the ether and alcohol to evaporate, as alcohol must be eliminated before starting the digestion with malt, or the charge may be dried at approximately 75° until the alcohol has been eliminated.)

Transfer as much of the dry material as possible from the filter paper into a glass mortar and pulverize all lumps. Transfer both filter paper and sample to a 500 cc volumetric flask, add 20–30 cc of $H_2O$, and thoroly wet the material by vigorous shaking.

Should more cold $H_2O$ be needed to make the material more fluid, calculate the quantity of hot $H_2O$ to be added accordingly, so that the total volume allowing for 40 cc of malt soln will not exceed 200 cc. Let stand a few min. and then add 100 cc of actively boiling $H_2O$. The sample now must be thoroly gelatinized at boiling temp. in a water bath.

Cool to 50° or lower, add 20 cc of the malt extract to controls as well as to charges, and place the flasks in a temp.-controlled water bath. Keeping the mash thoroly mixed, gradually raise the temp. to 70° in 20–30 min. Maintain at 70° for 30 min., stirring the mixture from time to time, then increase the temp. to 80°, and keep it at that temp. for 10 min. Finally heat to the boiling point. Keep the mixtures well stirred. Cool the contents of the flasks and the water bath to 55°. Add 20 cc of the malt extract, mix well, and hold at 55° for 1 hour, stirring about once every 10 min. At the termination of the digestion rapidly increase the temp. to above 80°.

Measure out 316 cc of 95% alcohol. Add a portion, a little at a time, to the contents of the flask, with thoro shaking between additions. After cooling to room temp. adjust the volume with $H_2O$ so that the quantity of liquid is 500 cc, making allowance for the volume occupied by the charge by adding 3 cc of $H_2O$ for every 4 g of charge present after bringing the contents to the 500 cc mark. The determination may be interrupted at this stage for several days. The volume should be readjusted if evaporation has occurred in the meantime. Mix thoroly, breaking up any ropy coagulum as much as possible by pouring back and forth from one large beaker to another. Filter thru dry paper. Test the solid residue for starch, either microscopically or by the I color test, after elimination of alcohol and gelatinization with $H_2O$. (If more than the merest trace of starch is found, reject the entire determination.) Evaporate exactly 200 cc of the filtrate on a steam bath to a volume of 15–20 cc, or until practically all alcohol has been expelled. Do not allow the evaporation to proceed to dryness.

Transfer the aqueous residue of starch conversion products to a 200 cc volumetric flask with hot $H_2O$, using a rubber-tipped rod to recover any dextrine that may be present. Allow to cool somewhat, and complete the volume to 200 cc. Transfer the contents to a suitable digestion flask, add 20 cc of HCl (sp. gr. 1.125) made by diluting 68 cc of strong acid (sp. gr. 1.19, or 37% HCl) to 100 cc and connect the flask with a reflux condenser. Heat in a boiling water bath for 2.5 hours. Cool, and for samples of linseed meal or other material yielding solns which at this stage need further purification, add not more than 1 cc of a 10% soln of phosphotungstic acid in 1% HCl. Mix, and allow to stand at least 15 min. Increase the volume with $H_2O$ to 250 cc in a volumetric flask, mix well, and filter thru dry paper. Partially neutralize 200 cc of the filtrate while stirring by adding 10 cc of a strong soln of caustic soda (44 g of NaOH per 100 cc of $H_2O$) and nearly complete the neutralization with a little powdered anhydrous $Na_2CO_3$. Transfer to a 250 cc flask with $H_2O$, cool to room temp., make up to the mark, and thoroly mix. Filter, if necessary, and determine the dextrose in a 50 cc aliquot of the filtrate, gravimetrically, as directed under **XXXIV, 59** or **61.** Correct the weight of dextrose obtained by subtracting the weight of dextrose found for the same aliquot of the malt control, and multiply the corrected weight of dextrose by 0.90 to obtain the weight of starch.

$$\text{Aliquots: Charge} \times \frac{200}{500} \times \frac{200}{250} \times \frac{50}{250}, \text{ or Charge} \times 0.064.$$

## PENTOSANS[9]—OFFICIAL

**28**

### REAGENTS

(**a**) *Hydrochloric acid.*—Contains 12% by weight HCl. To 1 volume of HCl add 2 volumes of $H_2O$. Determine the percentage of acid by titration against standard alkali and adjust to proper strength by dilution or addition of more strong acid, as may be necessary.

(**b**) *Phloroglucin.*—Dissolve a small quantity of phloroglucin in a few drops of acetic anhydride, heat almost to boiling, and add a few drops of $H_2SO_4$. A violet color indicates the presence of diresorcin. A phloroglucin which gives more than a faint coloration may be purified by the following method:

Heat in a beaker about 300 cc of the dilute HCl (**a**) and 11 g of commercial phloroglucin, added in small quantities at a time, stirring constantly until it is nearly dissolved. Pour the hot soln into a sufficient quantity of the same HCl (cold) to make the volume 1500 cc. Allow to stand at least overnight, preferably several days, to permit the diresorcin to crystallize. Filter immediately before using. A yellow tint does not interfere with its usefulness. In using, add the volume containing the required quantity of phloroglucin to the distillate.

**29**

### DETERMINATION

Place such a quantity of the sample, 2–5 g, that the weight of phloroglucide obtained shall not exceed 0.300 g, in a 300 cc distillation flask, together with 100 cc of the dilute HCl and several pieces of recently ignited pumice stone. Place the flask on a wire gauze; connect with a condenser; and heat, rather gently at first, but then regulating so as to distil over 30 cc in about 10 min. Pass the distillate thru a small filter paper. Replace the 30 cc distilled by a like quantity of the dilute acid, added by means of a separatory funnel in such a manner as to wash down the particles adhering to the sides of the flask, and continue the process until the distillate amounts to 360 cc. To the total distillate add gradually a quantity of phloroglucin dissolved in the dilute HCl and thoroly stir the resulting mixture. The quantity of phloroglucin used should be about double that of the furfural expected. The soln turns yellow, then green, and very soon there appears an amorphous greenish precipitate that grows darker rapidly, till it becomes almost black. Make the soln up to 400 cc with the dilute HCl and allow to stand overnight.

Collect the amorphous black precipitate in a weighed Gooch crucible having an asbestos mat, wash carefully with 150 cc of $H_2O$ so that the $H_2O$ is not entirely removed from the crucible until the very last, then dry for 4 hours at the temp. of boiling $H_2O$, cool, and weigh in a weighing bottle. The increase in weight is taken to be furfural phloroglucide. To calculate the furfural, pentose, or pentosan from the phloroglucide, use the following formulas given by Kröber:

(1) For a weight of phloroglucide, designated by "$a$" in the following formulas, *under* 0.03 g,

$$\begin{aligned}\text{Furfural} &= (a+0.0052)\times 0.5170.\\ \text{Pentoses} &= (a+0.0052)\times 1.0170.\\ \text{Pentosans} &= (a+0.0052)\times 0.8949.\end{aligned}$$

In the above and also in the following formulas, the factor 0.0052 represents the weight of the phloroglucide that remains dissolved in the 400 cc of acid soln.

(2) For a weight of phloroglucide "$a$" *between* 0.03 and 0.300 g, use Kröber's table, **XLII, 18**, or the following formulas:[10]

$$\begin{aligned}\text{Furfural} &= (a+0.0052)\times 0.5185.\\ \text{Pentoses} &= (a+0.0052)\times 1.0075.\\ \text{Pentosans} &= (a+0.0052)\times 0.8866.\end{aligned}$$

(3) For a weight of phloroglucide "$a$" *over* 0.300 g,

$$\begin{aligned}\text{Furfural} &= (a+0.0052)\times 0.5180.\\ \text{Pentoses} &= (a+0.0052)\times 1.0026.\\ \text{Pentosans} &= (a+0.0052)\times 0.8824.\end{aligned}$$

## 30 GALACTAN—TENTATIVE

Extract a convenient quantity of the sample, representing 2.5–3 g of the dry material, on a hardened filter with 5 successive portions of 10 cc of ether; place the extracted residue in a beaker, about 5.5 cm in diameter and 7 cm deep; add 60 cc of $HNO_3$ (sp. gr. 1.15); and evaporate on a steam bath to a volume of 20 cc. Let stand 24 hours, then add 10 cc of $H_2O$, and allow to stand another 24 hours. Pass thru a filter and wash the impure mucic acid crystals with 30 cc of $H_2O$ to remove as much of the $HNO_3$ as possible, and return filter and contents to the original beaker. Add 30 cc of $(NH_4)_2CO_3$ soln (consisting of 1 part $(NH_4)_2CO_3$, 19 parts $H_2O$, and 1 part of $NH_4OH$) and heat the mixture in a water bath, at 80°, for 15 min., with constant stirring. The $(NH_4)_2CO_3$ combines with the mucic acid, forming soluble $NH_4$-mucate. Wash the filter paper and contents several times with hot $H_2O$ by decantation, passing the washings thru a filter paper, to which finally transfer the residue, and wash thoroly. Evaporate the filtrate to dryness on a water bath, avoiding unnecessary heating which causes decomposition; add 5 cc of $HNO_3$ (sp. gr. 1.15); stir the mixture thoroly; and allow to stand for 30 min. Collect the precipitated mucic acid on a weighed Gooch crucible or other filter; wash with 10–15 cc of $H_2O$, then with 60 cc of 95% alcohol, and then a number of times with ether; dry at the temp. of boiling $H_2O$ for 3 hours; and weigh. Multiply the weight of the mucic acid by 1.33 to convert to galactose, and by 1.20 to convert to galactan.

## 31 WATER-SOLUBLE ACIDITY[11]—TENTATIVE

Weigh 10 g of the sample into a shaking bottle, add 200 cc of $H_2O$, and shake for 15 min. Filter the extract thru a folded filter and take a 20 cc aliquot (equivalent to 1 g of sample). Dilute with 50 cc of $H_2O$ and titrate with 0.1 $N$ NaOH soln, using phenolphthalein indicator. Report results in terms of cc of 0.1 $N$ NaOH required for neutralizing the extract from 1 g of material.

## SPECIAL METHODS

### 32 RICE HULLS IN RICE BRAN[12]—TENTATIVE

Thoroly mix the sample to be examined. Withdraw a small portion and grind until all passes thru a 60-mesh sieve. Weigh 4 mg on a slide ruled in parallel lines 1/20 inch apart or transfer to the ruled slide after weighing. Add just sufficient chloral hydrate soln (1+1) to fill in under the cover-glass, which, preferably, should be square (about 22 mm). After the cover-glass is in place warm gently, but do not boil, to eliminate the starch masses and clear the tissues. Count the particles of hull tissue, using a microscope having a magnification of about 90 diameters. The high refraction and yellowish green color of the hull particles will aid in distinguishing the small pieces in which the structure is not easily recognizable. In order to avoid duplicate counting, it is well to disregard the particles that extend over the upper line of the strip. Compare the results with those obtained on standards containing known quantities of hulls.

### 33 OAT HULLS IN OATS AND OAT FEEDS[13]—TENTATIVE

(Results are only approximate)

Place in a 100 cc beaker 800 cc of $H_2O$ and 2 g of the sample, previously ground to pass thru a sieve having circular openings 1 mm in diameter. Stir vigorously to obtain a centrifugal effect, allow to stand for 5 min. and then decant the supernatant liquid carefully, retaining so far as possible all hull particles. Repeat this procedure several times until the supernatant liquid becomes clear, or nearly so, and then transfer the residue with the aid of 150 cc of $H_2O$ to a 300 cc beaker. Add 5 drops of HCl and boil for 2 min., constantly stirring the mixture. Transfer to the original beaker with the aid of 500 cc of $H_2O$, stir, and allow to stand until the supernatant liquid is clear. Draw off the liquid by means of a siphon of rubber tubing having a 3 or 4 mm bore, using a pinch clamp to control the flow so that practically all the liquid may be siphoned off. (Tilting the beaker will also help to obtain this result.) If on standing a deposit forms in the siphonate, repeat the siphonation. Transfer the hulls with the aid of $H_2O$ to a paper filter, wash several times with alcohol, and allow to dry to constant weight at room temp. When dry, carefully remove the hulls from the paper, using if necessary a small stiff brush, and weigh. (A weighed Gooch crucible may be used instead of the paper filter.) Multiply the weight of hulls by 50 to obtain the percentage of hulls in the sample.

### 34 GRIT IN POULTRY AND SIMILAR FEEDS[12]—TENTATIVE

Place 2 g of the thoroly mixed sample, prepared as directed under **1**, in an evaporating dish of about 30 cc capacity. Add about 5 cc of $CHCl_3$ and mix gently with a glass rod so that the liquid comes in contact with all portions of the sample. Brush the particles adhering to the rod into the dish, and after pushing all particles down into the $CHCl_3$ with a 25 mm circular or square cover-glass, use the glass to skim off or pull the floating portion of the material over the top of the dish, taking care not to submerge the cover-glass deep enough to disturb the grit settled at the bottom of the dish. After skimming until the surface of the $CHCl_3$ is nearly clear, slowly pour the supernatant liquid into a second evaporating dish. Wash the sides of the dish with a few cc more of $CHCl_3$ and repeat the skimming and decanting operation until no floating particles remain. (This will require 10–15 cc of $CHCl_3$.) When grit only remains, allow the last traces of $CHCl_3$ to evaporate spontaneously, and weigh. Weight of residue times 50 equals the percentage of grit. After weighing, examine the residue for impurities. Also pour out the $CHCl_3$ washings collected in the second dish and observe whether any grit has been transferred to it during the process.

### 35 BONE IN MEAT SCRAP OR TANKAGE[14]—TENTATIVE

Separate the bone as directed under **34.** In some instances it may be found necessary, after the first washing with $CHCl_3$, to rub the remaining residue of bone with a glass rod or small pestle in order to bring some of the adhering particles to the surface of the $CHCl_3$.

### 36 CALCIUM OXIDE IN MINERAL FEEDS[15]—TENTATIVE

Grind the sample and take a 2 g portion, igniting in a muffle to burn off organic matter, if present. Dissolve the residue in dilute HCl with the aid of heat. Make up the dissolved material to 250 cc in a graduated flask, pipet 25 cc of the clear liquid into a 600 cc beaker, add $NH_4OH$ in slight excess, and dilute until the volume is about 100 cc. Instead of boiling and filtering off $Fe_2O_3$ and $Al_2O_3$ at this point, make the soln slightly acid with 0.1 *N* HCl, using methyl red indicator. Bring back with 0.1 *N* NaOH to neutral point as shown by the intermediate or brownish color of the indicator. Dilute to about 300 cc, boil, and add slowly with constant stirring a soln containing 3 g of $(NH_4)_2C_2O_4$, which should also be boiling hot. Let stand overnight to allow the precipitate to settle. Filter and wash thoroly with cold $H_2O$. Transfer the paper and the precipitate to a 600 cc beaker and dilute with $H_2O$ to about 300 cc. Add 5 cc of $H_2SO_4$. Stir well and bring the soln to boiling. While hot, titrate with 0.1 *N* $KMnO_4$ until a slightly pink color is obtained. Calculate the percentage of CaO in the sample.

### HYDROCYANIC ACID FORMED BY THE HYDROLYSIS OF GLUCOSIDES IN BEANS[16]

### 37 *I. Acid Titration Method—Tentative*

Grind the sample to pass a 20-mesh sieve. Introduce 10–20 g of the ground material into an 800 cc Kjeldahl flask, add 100 cc of $H_2O$, and macerate at room temp. for 2 hours. Add 100 cc of $H_2O$ and distil with steam, collecting the distillate in 20 cc of 0.02 *N* $AgNO_3$ soln acidified with 1 cc of $HNO_3$. Before distilling, adjust the apparatus so that the tip of the condenser dips below the surface of the liquid in the receiver. When 150 cc has passed over, filter the distillate thru a Gooch crucible; wash the receiver and Gooch with a little $H_2O$; and titrate the excess of $AgNO_3$ in the combined filtrate and washings with 0.02 *N* KCNS soln, using ferric alum indicator. 1 cc of 0.02 *N* $AgNO_3$ soln = 0.54 mg of HCN.

### 38 *II. Alkaline Titration Method—Tentative*

Macerate and distil 10–20 g of the sample as directed under **37** except to collect the distillate in a vessel containing 0.5 g of NaOH dissolved in 20 cc of $H_2O$. When 150 cc has psssed over, titrate the distillate with 0.02 *N* $AgNO_3$ soln, adding the standard soln slowly, dropwise, and stirring constantly until the first permanent turbidity appears. 1 cc of 0.02 *N* $AgNO_3$ soln = 1.08 mg of HCN.

### 39 *III. Prussian Blue Method—Tentative*

Macerate and distil 10–20 g of the sample as directed under **37,** using the NaOH soln in the receiver, and dilute the distillate to 200 cc in a volumetric flask. Concentrate 20 cc of this soln, which must contain a slight excess of NaOH, in a 200 cc round-bottomed flask attached to a vacuum pump and condenser, heating the flask in a water bath below 70°. (An adapter may be used to avoid loss by spattering.) When the volume has been reduced to 1 cc or less, add 0.2–0.5 cc of freshly prepared 3% $FeSO_4$ soln and about 0.5 g of KF. Exhaust the flask at once by means of a vacuum pump. Mix the contents by rotating the flask. After 5–10 min. detach the flask and acidify the mixture with 30% $HNO_3$. The blue color usually appears at once, altho in case traces only are present it is sometimes necessary to warm to about 50° in a water bath. Dilute the resulting suspension of Prussian blue to a convenient volume and compare the color with a standard Prussian blue mixture, prepared as directed above from a standard soln containing 1 mg of KCN diluted to 25 cc.

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[9] J. Landw., **48,** 357 (1900); **49,** 7 (1901).
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[11] Ibid., **137,** p. 152.
[12] J. Assoc. Official Agr. Chem., **5,** 77 (1921).
[13] Ibid., **9,** 149 (1926); **10,** 32 (1927).
[14] Ibid., **5,** 424 (1922).
[15] Ibid., **10,** 177 (1927).
[16] J. Am. Chem. Soc., **37,** 601 (1915); J. Assoc. Official Agr. Chem., **4,** 151 (1920).

# XXVIII. MEAT AND MEAT PRODUCTS

## MEAT

### 1 PREPARATION OF SAMPLE—OFFICIAL

To prevent loss of $H_2O$ during preparation and subsequent handling do not use small samples. Keep the ground material in glass or similar containers provided with air- and water-tight covers. Prepare samples for analysis in the following manner:

(**a**) *Fresh meats, dried meats, cured meats, smoked meats, etc.*—Separate as completely as possible from any bone; pass rapidly thru a food chopper 3 times, thoroly mixing after each grinding; and begin all determinations as soon as practicable. If any delay occurs, chill the sample to inhibit decomposition.

(**b**) *Canned meats.*—Pass the entire contents of a can thru a food chopper, as directed under (**a**).

(**c**) *Sausages.*—Remove from casings and pass thru a food chopper, as directed under (**a**).

Dry the portions of the samples under (**a**), (**b**), and (**c**) not needed for immediate analysis, either in vacuo below 60° or by evaporating on a steam bath 2 or 3 times with alcohol. Extract the fat from the dried product with gasoline (b. p. below 60°) and allow the gasoline to evaporate spontaneously, finally expelling the last traces by heating for a short time on a steam bath. Do not heat the sample or the separated fat longer than necessary because of tendency to decompose. Reserve the fat for examination as directed under **XXXI**, keeping it in a cool place and complete the examination before it becomes rancid.

### 2 MOISTURE—OFFICIAL

Proceed as directed under **XXVII, 2** or **6**, employing **6** when the dried sample is to be used for further determinations.

### 3 ADDED WATER IN SAUSAGE AND SIMILAR MEAT PRODUCTS[1]—TENTATIVE

(**a**) *Moisture.*—Weigh accurately about 10 g of the ground sample into a tared weighing bottle, approximately 2 inches in diameter, containing a short glass rod flattened at one end. Remove 2.5–3 g for the protein determination. Reweigh the remainder in the bottle, spreading it out in a thin layer over the sides and bottom by means of the glass rod, and use this sample for the determination of moisture. Dry in air at atmospheric pressure at a temp. of 101°–102° for approximately 16–18 hours, or at a temp. of approximately 125° (not lower than 120° nor higher than 130°) for approximately 2–3 hours, or until no significant loss of weight occurs on subsequent drying for a period of 1–2 hours. (If preferred, moisture may be determined according to **2**.)

(**b**) *Nitrogen.*—Determine total N as directed under **II, 17, 22,** or **24.** Calculate the protein by multiplying the total N by the factor 6.25.

(**c**) *Added Water.*—Multiply the percentage of protein calculated from the N determination by 4 and subtract the result from the percentage of moisture found. Report the difference, if any, as added $H_2O$.

### 4 ASH—OFFICIAL

Proceed as directed under **XXVII, 8.**

5

### CRUDE FAT OR ETHER EXTRACT—OFFICIAL

Proceed as directed under **XXVII, 15.**

6

### TOTAL PHOSPHORUS—OFFICIAL

Destroy the organic matter as directed under **II, 6** (c) or (d), and proceed as directed under **II, 7** or **10.**

7

### TOTAL NITROGEN—OFFICIAL[2]

Proceed as directed under **II, 19, 22,** or **24,** using about 2 g of the fresh sample.

## AMMONIA

### *Aeration Method*[3]*—Tentative*

8

### APPARATUS

Use the apparatus illustrated in Fig. 28. *A* is a wash bottle $\frac{1}{4}$ full of $H_2SO_4$ (1+9); *B* is a tube containing the sample; *C* is a rubber disk; and *D* is a 5 cc bulb to prevent spray from being carried over into the tube *E*, which contains the standard acid; *F* is a safety bottle.

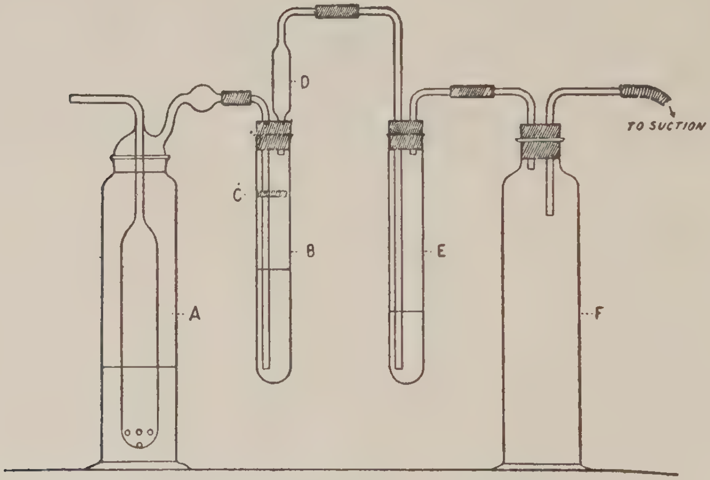

FIG. 28.—APPARATUS FOR THE DETERMINATION OF AMMONIA.

9

### DETERMINATION

Introduce 2–4 g of the finely divided meat into tube *B* and add 20 cc of $NH_3$-free $H_2O$. Place a measured quantity of 0.04 *N* or 0.02 *N* $H_2SO_4$ or HCl in tube *E*. Then add 1 cc of saturated K-oxalate soln to the sample in tube *B*, introduce a few drops of kerosene, and finally add just sufficient saturated Na- or K-carbonate soln to render the mixture alkaline. Place the tubes in position at once; pass air thru the apparatus; and titrate the standard acid in tube *E* at hourly intervals until $NH_3$ ceases to be given off, using methyl-red, cochineal, or congo red indicator. (If preferred, the $NH_3$ collected in tube *E* may be determined by nesslerizing as directed under **XXXVII, 11.**)

## NITRATES (INCLUDING ALSO NITRITES)

*Ferrous Chloride Method*[4]*—Tentative*

### 10 REAGENTS

(a) *Ferrous chloride soln.*—Dissolve 400 g of nails, tacks, or other small pieces of iron in a 2 liter Florence flask with 1 liter of HCl, excluding the air from the flask by means of a stopper equipped with a Bunsen valve. When the evolution of gas ceases, transfer, and keep the soln in completely filled 50 cc glass-stoppered bottles. Use only freshly opened bottles of the reagent for the determination.

(b) *Standard sodium nitrate soln.*—Dissolve 2 g of $NaNO_3$ in 1 liter of recently boiled $H_2O$. Determine NO in 50 cc of this soln (equivalent to 0.1 g of $NaNO_3$) as directed under **12.**

### 11 APPARATUS

Clamp a 500 cc Kjeldahl flask fitted with a 2-holed stopper to an iron stand. Thru one of the holes pass the stem of a 100–125 cc cylindrical separatory funnel having a glass stopcock, and into the other fit a delivery tube leading downward at an angle from the flask into a trough containing a strong soln of commercial NaOH (1+1). Terminate the upper end of the delivery tube just below the stopper in the flask and place the lower end, which is slightly constricted, bent upward, and covered with rubber tubing to prevent fracture, under the surface of the strong NaOH soln in the trough, the exit being just below the mouth of an inverted measuring tube (50 cc plain eudiometer tube) filled with a strong soln of commercial NaOH. A single coil of tin tubing fitted into the trough and carrying a current of cold $H_2O$ greatly facilitates the determination.

### 12 DETERMINATION

Extract 100 g of the sample by boiling 6 to 7 times with successive 35–50 cc portions of $H_2O$, decant the extracts thru a muslin or paper filter into a casserole, and evaporate the combined extracts to a volume of about 50 cc. Introduce 50 cc of the $FeCl_2$ soln and 50 cc of HCl (1+2.5) into the Kjeldahl flask, close the stopcock of the funnel, move the end of the delivery tube so that the escaping air will not pass into the measuring tube, and boil the contents of the flask until the air is completely expelled. Place the exit end of the delivery tube beneath the measuring tube and boil the contents of the flask 1 min. longer to make certain that no air remains. Introduce the concentrated extract of the sample into the flask, a little at a time thru the funnel, continually boiling the contents of the flask to force the NO gas into the measuring tube. Finally rinse the casserole and the funnel 3 or 4 times with 5–10 cc of recently boiled $H_2O$, adding the rinsings to the contents of the evolution flask in the manner described above. When the evolution of gas ceases, cover the opening of the measuring tube with a porcelain crucible, using tongs, and carefully transfer the tube to a tall glass jar containing a strong soln of commercial NaOH (1+1), kept at room temp. The temp. of the surrounding caustic soln will soon (10–15 min.) be imparted to the contents of the tube, and the volume of NO is read with the tube in such a position that the level of the solns within the tube coincides with the level outside. Calculate the percentage of nitrates and nitrites as $NaNO_3$ from the volume of NO obtained from the sample compared with the volume obtained from 0.1 g of $NaNO_3$, both measured under identical conditions.

After the measuring tube has been removed, quickly insert another filled with a strong soln of commercial NaOH (1+1) over the delivery tube and boil 1 min. longer to make sure that all the NO has been expelled. Run another 50 cc portion of the standard soln into the apparatus and repeat the determination. Then run the samples in the same manner, in each case making certain that all the NO gas has been expelled. After 6 to 8 determinations are made, excluding the 2 standards, finally run another standard. The 3 standards should check within 0.5 cc on about 30–35 cc; 0.1 g of $NaNO_3$ should give 26.36 cc of NO at 0° and 760 mm pressure. Report results as percentage of $NaNO_3$.

### *Phenoldisulfonic Acid Method*[5]*—Tentative*

#### 13 REAGENTS

(a) *Phenoldisulfonic acid soln.*—Heat 6 g of phenol with 37 cc of $H_2SO_4$ on a steam bath, cool, and add 3 cc of $H_2O$.

(b) *Standard comparison soln.*—Dissolve 1 g of pure, dry $NaNO_3$ in $H_2O$ and dilute to 1 liter. Evaporate 10 cc of this soln to dryness on a steam bath, add 2 cc of the phenoldisulfonic acid soln, mix quickly and thoroly by means of a glass rod, heat for about a minute on a steam bath, and dilute to 100 cc. 1 cc of the diluted soln = 0.1 mg of $NaNO_3$. Prepare a series of standard comparison tubes by introducing quantities ranging from 1 to 20 cc of the diluted soln (0.1–2.0 mg of $NaNO_3$) into 50 cc Nessler tubes, adding 5 cc of $NH_4OH$ to each and diluting to 50 cc. The standard tubes thus prepared are permanent for several weeks if kept tightly stoppered.

#### 14 DETERMINATION

Weigh 1 g of the sample into a 100 cc flask; add 20–30 cc of $H_2O$; and heat on a steam bath for 15 min., shaking occasionally. Add 3 cc of a saturated nitrate-free $(Ag)_2SO_4$ soln for each per cent of NaCl present, then 10 cc of basic Pb-acetate soln and 5 cc of alumina cream, shaking after each addition. Make up to the mark with $H_2O$; shake; and filter thru a folded filter, returning the filtrate to the filter until it runs thru clear. Evaporate 25 cc of the filtrate to dryness; add 1 cc of the phenoldisulfonic acid soln; mix quickly and thoroly by means of a glass rod; add 1 cc of $H_2O$ and 3 or 4 drops of $H_2SO_4$; and heat on a steam bath for 2–3 min., being careful not to char the material. Then add about 25 cc of $H_2O$ and an excess of $NH_4OH$, transfer to a 100 cc volumetric flask, add 1–2 cc of alumina cream if not perfectly clear, dilute to volume with $H_2O$, and filter. Fill a 50 cc Nessler tube to the mark with the filtrate and determine the quantity of $NaNO_3$ present in the sample by comparison with the standard comparison tubes. If the soln is too dark for comparison with the standards, dilute with $H_2O$, and correct the result accordingly. Report as percentage of $NaNO_3$.

### NITRITES—TENTATIVE

#### 15 (Applicable to Cured Meats.)

Weigh 5 g of the finely comminuted and thoroly mixed sample into a 50 cc beaker. Add approximately 40 cc of nitrite-free $H_2O$ heated to a temp. of 80°. Mix thoroly by stirring with a glass rod, taking care to break up all lumps, and transfer to a 500 cc graduated flask. Wash out the beaker and rod thoroly with successive portions of the hot $H_2O$, adding all washings to the flask. Add sufficient hot $H_2O$ to bring the contents of the flask to a volume of approximately 300 cc, transfer the flask to the steam bath, and let stand for 2 hours, shaking occasionally. Add 5 cc of saturated $HgCl_2$ soln and mix. Cool to room temp., make up to the mark with nitrite-free $H_2O$, and mix again. Filter, and determine nitrite N in a suitable aliquot as directed under **XXXVII, 15,** reporting results as parts of $NaNO_2$ per million.

## STARCH

(In chopped meat, sausage, deviled meat, etc.)

**16**

### *Qualitative Test—Tentative*

Treat 5–6 g of the sample with boiling $H_2O$ for 2–3 min., cool the mixture, and test the supernatant liquid with I soln [**XXXIII, 20** (j)]. (In interpreting this test it should be remembered that a small quantity of starch may be present as the result of the use of spices. If a marked reaction is given, however, it may be concluded that starch or flour has been added, and a quantitative determination should be made. The qualitative test may be replaced by a microscopic examination, which discloses not only the presence of added starch but also the variety used.)

**17**

### *Quantitative Method[7]—Tentative*

Treat in a 200 cc beaker 10 g of the finely divided sample with 75 cc of an 8% soln of KOH in 95% alcohol and heat on a steam bath until all the meat is dissolved (30–45 min.). Add an equal volume of 95% alcohol, cool, and allow to stand for at least an hour. Filter by suction thru a thin layer of asbestos in a Gooch crucible. Wash twice with a warm 4% soln of KOH in alcohol, 50% by volume, and then twice with warm 50% alcohol. Discard the washings. Retain as much of the precipitate in the beaker as possible until the last washing. Place the crucible with contents in the original beaker and add 40 cc of $H_2O$ and 25 cc of $H_2SO_4$. Stir during the addition of the acid and make sure that the acid comes in contact with all the precipitate. Allow to stand about 5 min.; add 40 cc of $H_2O$; and heat just to boiling, stirring constantly. Transfer the soln to a 250 cc volumetric flask, add 2 cc of 20% phosphotungstic acid soln, allow to cool to room temp., and make up to the mark with $H_2O$. Filter thru a starch-free filter paper; pipet 100 cc of the filtrate into a 200 cc volumetric flask; neutralize with 10% NaOH soln; make up to volume; and determine the dextrose present in a 50 cc portion of the filtrate as directed under **XXXIV, 38,** titrating the $Cu_2O$ precipitate as directed under **XXXIV, 41.** Weight of dextrose $\times 0.9 =$ weight of starch.

## GLYCOGEN

**18**

### *Qualitative Test[8]—Tentative*

Boil 50 g of the macerated sample with 50 cc of $H_2O$ for 15–30 min. Filter the broth thru moistened filter paper or fine linen. To a portion of the filtrate in a test tube add a few drops of a mixture of 2 parts of I, 4 parts of KI, and 100 parts of $H_2O$. If a considerable quantity of glycogen is present, it produces a dark brown color; this color is destroyed by heating, but it reappears on cooling. If starch is present, it may be precipitated by treating the water extract with two volumes of glacial acetic acid and after filtering applying the test for glycogen to the filtrate.

### *Quantitative Method[9]—Tentative*

**19**

#### PREPARATION OF SOLUTION

Weigh by difference about 25 g of the finely ground and thoroly mixed sample. Place in a 400 cc beaker and mix with 50 cc of KOH soln (1.5+1), free from carbonate. Cover the beaker with a watch-glass and digest on a steam bath for 2 hours, stirring occasionally. At the end of the 2 hours, dilute to approximately 200 cc with cold $H_2O$.

### 20 DETERMINATION

Add to the soln under **19** an equal volume of 95% alcohol, cover with a watch-glass, and set aside for 10–12 hours. Decant the supernatant liquid thru a folded 18.5 cm filter, allowing the glycogen to remain in the beaker, and wash by decantation with 66% alcohol (2 volumes of 95% alcohol + 1 of $H_2O$) until the glycogen is white, or nearly so. (Usually about 4 washings are required.) Transfer the washed precipitate from the beaker to the filter and wash 2 or 3 times with the 66% alcohol. (The soln filters slowly, and the funnel should be covered with a watch-glass to prevent excessive evaporation. The albuminous substance present retards the filtration if it is permitted to dry on the paper. If the washing by decantation is not made as complete as possible, it will be difficult to obtain the glycogen free from the coloring matter.)

After the washing is completed, close the bottom of the funnel by a piece of rubber tubing and a pinch-cock. Fill the funnel with warm $H_2O$, cover with the watch-glass and let stand 2–3 hours, or overnight. Open the pinch-cock and allow all the soln to pass thru the filter into a beaker. Close the funnel with the pinch-cock and fill with warm $H_2O$ as before. Allow this $H_2O$ to remain in the funnel for 1 hour and then filter as before. At first the glycogen soln appears quite turbid. Continue washing with warm $H_2O$ until the filtrate becomes perfectly clear. To the soln of glycogen in $H_2O$, add double its volume of 95% alcohol and let stand overnight to complete the reprecipitation of the glycogen. Filter, and wash as before with 66% alcohol.

If desired, the last filtration may be made thru a weighed Gooch crucible and the weight of glycogen may be determined after drying to constant weight. This gives results that are approximately correct. More satisfactory results are obtained by hydrolyzing the glycogen with dilute HCl and determining the resultant dextrose. Dissolve the glycogen on the filter in warm $H_2O$ as directed above, collecting the filtrate and washings in a 300 cc volumetric flask and keeping the volume within 225 cc. Add 12.5 cc of HCl to the combined filtrate and washings, mix, and place in a boiling water bath for 3 hours. Cool, neutralize with 10% NaOH soln, cool again, make up to volume with $H_2O$, and determine dextrose in an aliquot of the soln as directed under **XXXIV, 61**, determining the reduced Cu as directed under **XXXIV, 41.** The corresponding weight of dextrose $\times 0.9 =$ its equivalent of glycogen. Correct this result for dilution to obtain the percentage of glycogen in the sample.

## SUGAR—TENTATIVE

### 21 REAGENT

*Phosphotungstic acid soln.*—Dissolve 100 g of phosphotungstic acid in $H_2O$ and dilute to 100 cc.

### 22 DETERMINATION

Weigh 100 g of the finely ground sample into a 600 cc beaker, add 200 cc of $H_2O$, heat to boiling, and boil gently for 5 min. Stir the contents of the beaker frequently during this and subsequent extractions to prevent bumping. (When several samples are extracted at the same time a mechanical stirring device is practically a necessity.) Remove the beaker from the flame, allow the insoluble matter to settle, and decant the clear liquid on an asbestos mat in a 4-inch funnel. Filter with the aid of suction. Add 150 cc of hot $H_2O$ to the residue in the beaker, boil gently for 5 min., let settle, and decant the clear liquid as above. Repeat the operation, finally transfer the contents of the beaker to the funnel, wash with 150–200 cc of hot $H_2O$, and press the meat residue as dry as possible. Transfer the contents of the filter flask to an evaporating dish and evaporate on a steam bath to a volume of about 25 cc but not to dryness. Transfer the extract to a 100 cc volumetric flask, taking care that the volume of liquid does not exceed 60 cc. Add 25–35 cc of the phosphotungstic acid soln, shake vigorously, let stand a few min. for gas bubbles to rise to the surface, make to volume, shake, and either filter or centrifugalize. (The use of a centrifuge is to be preferred, because a larger volume of liquid is obtained.) Test a portion of the filtrate with dry phosphotungstic acid for complete precipitation. If an appreciable precipitate forms, take an aliquot of the filtrate, add 5-10 cc of the phosphotungstic acid soln, make to volume, filter, and test the filtrate for complete precipitation. The filtrate should also show not more than a slight reaction for creatinin when tested by adding to 5 cc a few drops of a saturated aqueous soln of picric acid and making the mixture alkaline with a few drops of 10% NaOH soln.[10]

Transfer 50 cc of the clarified extract to a 100 cc volumetric flask, add 5 cc of HCl, and invert the soln as directed under **XXVII, 23**. Cool the soln, neutralize to litmus, cool, make to volume, and filter. To the filtrate add sufficient dry powdered KCl to precipitate the excess of phosphotungstic acid, filter, test the filtrate for complete precipitation, and determine the reducing sugar as directed under **XXXIV, 35, 38,** ascertaining the quantity of reduced Cu as directed under **XXXIV, 42.** Calculate the total sugar as dextrose.

If an abnormal reduction is obtained when the clarified meat extract is boiled with Fehling's soln, i.e., if the soln turns yellow, brown, green, or muddy in appearance instead of reddish-blue, discard the determination, since incomplete precipitation of the nitrogenous compounds, due to the use of insufficient phosphotungstic acid, is indicated.

### 23 PRESERVATIVES—OFFICIAL

Proceed as directed under **XXXII.**

### 24 METALS—TENTATIVE

Proceed as directed under **XXIX.**

### 25 COLORING MATTERS—TENTATIVE

Proceed as directed under **XXI.**

## SOLUBLE AND INSOLUBLE NITROGEN—TENTATIVE

### 26 PREPARATION OF SOLUTION

Exhaust 7–25 g of the sample (depending upon the water content) in the following manner: Weigh into a 150 cc beaker, add 5–10 cc of cold (15°) $NH_3$-free $H_2O$, and stir to a homogeneous paste. Then add 50 cc of cold $H_2O$; stir for 15 min. at 3 min. intervals; let stand for 2–3 min.; and decant the liquid thru a quantitative filter, collecting the filtrate in a 500 cc volumetric flask. Drain the beaker, pressing out the liquid from the meat residue by the aid of a glass rod. Add to the residue in the beaker 50 cc of cold $H_2O$; stir for 5 min.; and, after allowing to stand 2–3 min., decant as before. If a considerable portion of the meat is transferred to the filter, return it to the beaker by means of a glass rod. Repeat the extractions, using two 50 cc portions and four 25 cc portions of cold $H_2O$. After the last extraction transfer the entire insoluble portion to the filter and wash with three 10 cc portions of $H_2O$, allowing the material to drain thoroly after each addition of $H_2O$. Dilute to the mark and mix thoroly.

### 27 DETERMINATION

Determine the total N in a 50 cc aliquot of the soln obtained under **26**, proceeding as directed under **II, 19, 22** or **24.** Total N − soluble N = insoluble N. Insoluble protein = insoluble N × 6.25.

## 28 COAGULABLE NITROGEN—TENTATIVE

(For uncooked meat only)

Measure 150 cc of the extract from **26** into a 250 cc beaker and evaporate to 40 cc on a steam bath, stirring occasionally. Neutralize to phenolphthalein, add 1 cc of 0.1 *N* acetic acid, and boil gently for 5 min. (The coagulum should separate at once, leaving a clear liquid.) Filter thru a quantitative paper and wash the beaker thoroly 4 times with hot $H_2O$, taking special care to clean the sides. Finally wash the coagulum on the filter 3 times; dilute the combined filtrate and washings to a definite volume; and reserve for the determination of proteoses, peptones, and gelatin, **29**, and creatin, **31**. Transfer the coagulum with the paper to a Kjeldahl flask and remove, with $H_2SO_4$, any of the material adhering to the beaker, taking the usual 25 cc of acid in 5 cc portions for this purpose, heating the acid in the beaker on a hot plate, and rubbing with a glass rod. Proceed as directed under **7.** N obtained × 6.25 = percentage of coagulable proteins.

## PROTEOSE, PEPTONE, AND GELATIN NITROGEN

### 29 *Modified Tannin-Salt Method*[11]*—Tentative*

Transfer a 50 cc aliquot of the filtrate obtained under **28** to a 100 cc volumetric flask, add 15 g of NaCl and 10 cc of cold $H_2O$, shake until the NaCl has dissolved, and cool to 12°. Add 30 cc of 24% tannin soln cooled to 12°, dilute to the mark with $H_2O$ previously cooled to 12°, shake, and allow the mixture to stand at a temp. of 12° for 12 hours, or overnight. Filter at 12°, transfer 50 cc of the filtrate to a Kjeldahl flask, and add a few drops of $H_2SO_4$. Place the flask in a steam bath, connect with a vacuum pump, and evaporate to dryness. Determine N in the residue as directed under **II, 19,** using 30 cc of $H_2SO_4$ for the digestion. Conduct a blank determination, using the same quantity of reagents, and correct the result accordingly. Multiply the corrected result by 2 and deduct the quantity of N found from the N determined in another 50 cc aliquot of the filtrate from the coagulable N, **28,** without the tannin-salt treatment; the difference ×6.25 = the percentage of proteoses, peptones, and gelatin.

## 30 MEAT BASES—TENTATIVE

Deduct from the percentage of total N, **7**, the sum of the percentages of N obtained in the determination of insoluble N, **27,** coagulable N, **28,** and proteoses, peptones, and gelatin, **29,** to obtain the percentage of N of the meat bases. Multiply the result by 3.12 to obtain the percentage of meat bases.

## 31 CREATIN—OFFICIAL

Evaporate an aliquot or the remaining portion of the filtrate and washings from the coagulable N, **28,** (a portion having been used in **29**) to 5–10 cc; transfer with a minimum quantity of hot $H_2O$ to a 50 cc volumetric flask, keeping the volume below 30 cc; add 10 cc of 2 *N* HCl; and mix. Hydrolyze in an autoclave at 117–120° for 20 min., allow the flask to cool somewhat, remove, and chill under running $H_2O$. Partially neutralize the excess of acid by adding 7.5 cc of 10% NaOH soln, free from carbonates; dilute to the mark; and mix. Make a preliminary reading on 20 cc with a Duboscq colorimeter to ascertain the volume to use to obtain a reading of approximately 8 mm. Transfer such a volume of the soln to a 500 cc volumetric flask and add 10 cc of 10% NaOH soln and 30 cc of saturated picric acid soln (1.2%). Mix, rotate for 30 seconds, and let stand exactly 4.5 min. Dilute to the mark at once with $H_2O$; shake thoroly; and read in a Duboscq colorimeter set at 8 mm, comparing the color with 0.5 *N* $K_2Cr_2O_7$ soln.

If the reading is too high or too low (above 9.5 or below 7 mm), calculate the quantity necessary to obtain a reading of about 8 mm. The strength of the dichromate soln used must be checked against a standard creatin soln. Divide 81 by the reading and multiply by the volume factor to obtain the mg of creatinin; this value, multiplied by 1.16, gives creatin, which divided by the weight of the sample and mulitplied by 100 gives the percentage of creatin.

The use of Kober's shade and the painting of the plunger, suggested for this nephelometer, assists in getting a sharper end point, relieves the eye strain, and may be employed if desired.

Example.—Twenty g of meat is extracted with $H_2O$ as directed under **26**, and the extract is diluted to 500 cc; 150 cc of this latter soln (equivalent to 6 g of meat) is treated as directed under **28**. The filtrate thus obtained is then evaporated and hydrolyzed as above and diluted to 50 cc; 25 cc of this last soln is treated with NaOH soln and picric acid soln as directed above and diluted to 500 cc. This latter soln gives a Duboscq reading of 9 mm.

$$\frac{81}{9}\times\frac{50}{25}=\text{mg of creatinin;}$$

$$\frac{0.018\times1.16\times100}{6}=0.35\%\text{ creatin.}$$

## AMINO NITROGEN

### *Van Slyke Method*[12]*—Tentative*

**32** REAGENTS

(a) *Alkaline permanganate soln.*—Dissolve 50 g of $KMnO_4$ and 25 g of KOH in $H_2O$ and dilute to 1 liter.

(b) *Sodium nitrite soln.*—Dissolve 30 g of $NaNO_2$ in $H_2O$ and dilute to 100 cc.

(c) *Glacial acetic acid.*

**33** APPARATUS

Use the apparatus shown in Figs. 29 and 30, the former illustrating the manner in which the entire apparatus is arranged and the latter showing the details of the deaminizing bulb and connections. The Hempel gas pipet is filled with the alkaline permanganate soln.

**34** DETERMINATION

Fill with $H_2O$ the buret (*F*), the capillary tube leading to the Hempel pipet, and also the other capillary as far as *c*. Introduce into *A* sufficient glacial acetic acid to fill $\frac{1}{5}$ of *D*, etching the tube *A* with a mark to measure this quantity. Allow the acid to run into *D*, and turn cock *c* so as to allow the air to escape from *D*. Pour the $NaNO_2$ soln into *A* until *D* is full of soln and enough excess is present to rise a little above the cock into *A*. *A* is also marked for measuring off this quantity. Then close the gas exit from *D* at *c*, and, *a* being open, shake *D* for a few seconds until the liquid is forced down to the 20 cc mark in *D*. Then close *a*, open *c*, and shake the apparatus rapidly with the motor for 2 min., these operations being for the purpose of expelling all the air from *D*. Then turn *c* and *f* so that *D* and *F* are connected.

Measure off in *B* 10 cc or less, as the case may be, of the soln of the sample containing not more than 20 mg of amino N (about 1–2 g of the sample in the case of meat extracts) and allow it to run into *D*. Connect *D* with the motor as shown in Fig. 29 and shake for 5 min.

If the soln of the sample is viscous and threatens to foam over, rinse out *B*, and then thru it introduce a little caprylic alcohol into *D*, or, if it is known beforehand that the sample will cause excessive foaming, introduce a little caprylic alcohol into *D* thru *B*, rinsing *B* with alcohol and ether or drying with a roll of filter paper before adding the soln of the sample.

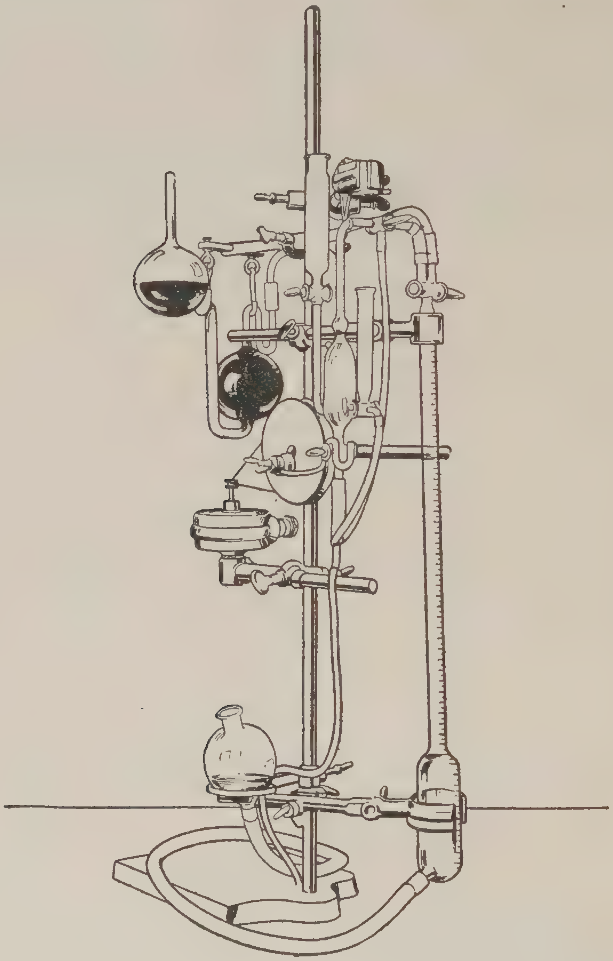

FIG. 29.—VAN SLYKE APPARATUS FOR THE DETERMINATION OF AMINO NITROGEN

During the shaking there is an evolution of N mixed with NO, the gases being collected in *F*. Force all the gas in *D* into *F* by opening *a* and filling *D* with liquid from *A*. Connect *F* with the Hempel pipet and force the gas into the latter by means of the leveling bulb, allowing the cock *a* to remain open during this and the succeeding operation in order to permit displacement of the liquid in *D* by the NO formed in the interval. Connect the driving rod with the pipet by lifting the hook from the shoulder of *D* and placing the other hook, on the opposite side of the driving rod, over the horizontal lower tube of the pipet. Shaking the pipet rather slowly for a few min. completes the absorption of NO except with the almost completely exhausted permanganate solns. Return the gas to the buret; adjust the level with the leveling bulb; note the volume of N, the temp., and the barometric pressure; and calculate the volume of N under standard conditions of temp. and pressure. Obtain the corresponding weight of N, divide the latter by 2, and from the quotient calculate the apparent percentage of amino N in the sample. Correct the result for a blank test performed as above, using 10 cc of $H_2O$ instead of the soln of the sample. The quantity of gas obtained in the blank is usually 0.3–0.4 cc, and nitrite solns giving a much large correction should be rejected.

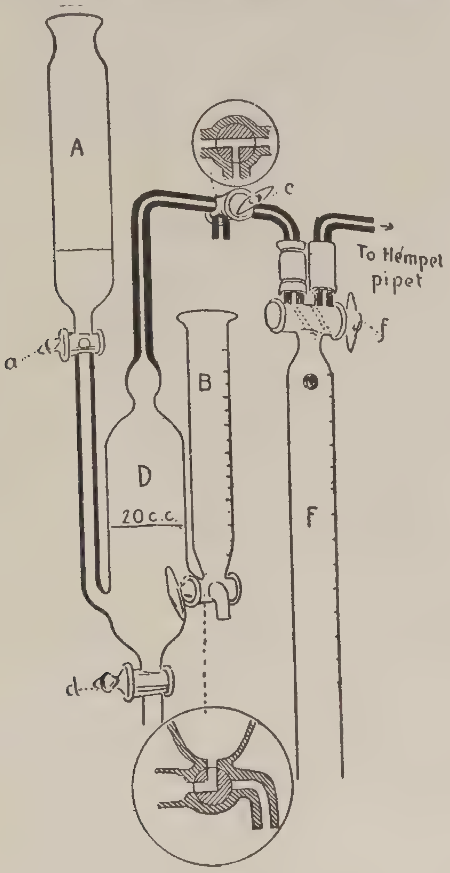

FIG. 30.—DETAILS OF THE DEAMINIZING BULB AND CONNECTION

With beef extracts and similar preparations, 5 min. is sufficient time to allow for the completion of the reaction in *D*. In general, the same time serves for the decomposition of alpha-amino acids, but with ammonia, methylamine, and most amines other than alpha-amines 1–1.5 hours should be allowed. For determinations on such substances mix the soln of the sample with the reagents, as described above, allow the mixture to stand in the apparatus till the end of the required time, and conclude the reaction by shaking the apparatus with the motor for 2–3 min. Continue the determination from this point as directed above.

### 35 *Sörenson Method*[13]—*Tentative*

To 20 cc of the filtrate from **28**, neutralized to phenolphthalein with $Ba(OH)_2$ or NaOH, or to 20 cc of an equivalent extract of the meat (in some cases a larger volume may be necessary) add 10 cc of a freshly prepared phenolphthalein-formol mixture (50 cc of commercial formol containing 1 cc of a 0.5% soln of phenolphthalein in 50% alcohol, exactly neutralized with 0.2 *N* $Ba(OH)_2$ or NaOH). Titrate the mixture with 0.2 *N* $Ba(OH)_2$ soln until a distinct red color appears, add a slight known excess of 0.2 *N* $Ba(OH)_2$, and titrate back to neutrality with 0.2 *N* HCl. Conduct a blank titration with the same reagents, using 20 cc of $H_2O$ in place of the soln to be tested. From the quantity of 0.2 *N* $Ba(OH)_2$ required to neutralize the mixture, corrected for the quantity used in the blank titration, calculate the quantity of amino N present (including $NH_3$ if this has not been removed). 1 cc of 0.2 *N* $Ba(OH)_2$ soln = 2.8 mg of amino N.

### 36 TOTAL SOLUBLE PHOSPHORUS—TENTATIVE

Evaporate to dryness 50 cc of the water extract prepared under **26**, moisten the residue with 10 cc of $H_2SO_4$, add a few drops of $HNO_3$, and heat on a hot plate until all the organic matter is destroyed. Add 100 cc of $H_2O$, boil for a few minutes, and proceed as directed under **II, 7**.

### 37 SEPARATION OF SOLUBLE INORGANIC AND ORGANIC PHOSPHORUS—TENTATIVE

To 500 cc of the extract prepared as directed under **26**, add 50 cc of magnesia mixture [**II, 5 (c)**], and stir thoroly. Allow to stand 15 min., add 25 cc of $NH_4OH$, cover, and allow to stand 3 days. Filter, and wash the precipitate with $NH_4OH$ (1+9). Dissolve the precipitate on the filter paper and that remaining in the beaker in dilute $HNO_3$ (1+1) and hot $H_2O$, receiving the soln in a 400 cc beaker. Neutralize with $NH_4OH$, make slightly acid with $HNO_3$, add 5 g of $NH_4NO_3$, and determine P as directed under **II, 7**.

## MEAT EXTRACTS AND SIMILAR PRODUCTS

### 38 PREPARATION OF SAMPLE—OFFICIAL

Remove liquid and semi-liquid meat extracts and similar preparations from the container and mix thoroly before sampling. (A little heating expedites the mixing of pasty extracts.) Carefully remove the sediment that forms in many liquid preparations from the bottom of the container and include in the sample. If the sample is in the form of cubes, grind 10–12 of the cubes in a mortar.

### 39 MOISTURE—OFFICIAL

Proceed as directed under **XXVII, 2**, using about 2 g of powdered preparations, about 3 g of pasty preparations, and 5–10 g of liquid extracts, according to the solid content. Dry the powdered preparations directly without admixture. Dissolve the pasty preparations in $H_2O$ and dry with sufficient ignited sand, asbestos, or pumice stone to absorb the soln. When glycerol is present, proceed as directed under **XXVII, 6**.

### 40 ASH—OFFICIAL

Proceed as directed under **XXVII, 8**. Add sufficient $H_2O$ to pasty preparations to effect soln and evaporate to dryness in order that the solids may be distributed evenly over the bottom of the dish.

### 41 TOTAL PHOSPHORUS—OFFICIAL

Destroy organic matter as directed under **II, 6** (c) or (d), and proceed as directed under **II, 7** or **10.**

### 42 CHLORIDES—OFFICIAL

Dissolve about 1 g of the sample, prepared as directed under **38,** in 20 cc of 5% $Na_2CO_3$ soln and proceed as directed under **XII, 33.**

### 43 FAT—TENTATIVE

Transfer the residue from the determination of moisture to a continuous extraction apparatus and proceed as directed under **XXVII, 15.**

### 44 TOTAL NITROGEN—OFFICIAL

Proceed as directed under **II, 19, 22** or **24.**

### 45 AMMONIA—TENTATIVE

Introduce 1 g of pasty extracts or 2–3 g of fluid extracts into tube *B* of the Folin apparatus and proceed as directed under **9.**

### 46 INSOLUBLE NITROGEN[14]—TENTATIVE

Dissolve in cold $H_2O$ 5 g of powdered preparations, 8–10 g of pasty extracts, and 20–25 g of fluid extracts. Filter, and wash with cold $H_2O$. Transfer the filter paper and contents to a Kjeldahl flask and determine N as directed under **II, 19, 22,** or **24.** If a large quantity of insoluble matter is present, transfer the weighed sample to a volumetric flask, dilute to a definite volume, shake thoroly, filter thru a folded filter, and determine N in an aliquot of the filtrate. Total N, **44,** $-$N in the total filtrate $=$ N in the insoluble N. Insoluble N $\times 6.25 =$ percentage of insoluble protein.

### 47 COAGULABLE NITROGEN—TENTATIVE

Prepare a soln of the sample as directed under **46.** Use as large an aliquot of the filtrate from the insoluble N, **46,** as practicable, and neutralize to phenolphthalein by the addition of acetic acid or NaOH, whichever may be necessary; add 1 cc of 1 *N* acetic acid, boil for 2–3 min., cool to room temp., dilute to 500 cc, and pass thru a folded filter.

Determine N in 50 cc of the filtrate as directed under **II, 19, 22** or **24.** Soluble N (total N $-$ N occurring as insoluble N) $- 10 \times$ the N obtained $=$ the percentage of N present as coagulable N. Coagulable N $\times 6.25 =$ coagulable protein in the sample.

### 48 PROTEOSES AND GELATIN[15]—TENTATIVE

Evaporate the filtrate from **47** to a small volume and saturate with $ZnSO_4$ (about 85 g to 50 cc, avoiding such an excess as would later cause bumping). Let stand several hours, filter, and wash the precipitate with saturated $ZnSO_4$ soln. Place the filter and precipitate in a Kjeldahl flask and determine N as directed under **II, 19, 22** or **24.** Or, if the precipitate is voluminous, which is unusual, dilute to a definite volume with saturated $ZnSO_4$ soln; filter; and determine the N in an aliquot of the filtrate as directed under **II, 19, 22** or **24.** N in the filtrate from the coagulable N, **47,** $-$ the N thus obtained $=$ the N of the precipitated protein (proteoses and gelatin).

### 49 GELATIN—TENTATIVE

Prepare a 50% soln of the sample, using hot $H_2O$, allow to cool, and place in an ice box for 2 hours. If gelatin is present, the soln will set.

The ratio of total creatinin to total N in a normal meat extract (1:1.5) assists in determining the presence of gelatin or gelatin derivatives. The ratio is decreased when gelatin or gelatin derivatives are present in any considerable quantity.

### 50 AMINO NITROGEN—TENTATIVE

Proceed as directed under **34** or **35**, using an aliquot of the filtrate from **47**.

### 51 ACID ALCOHOL-SOLUBLE NITROGEN[16]—TENTATIVE

Transfer 10 cc of an aqueous soln of the sample (10 g of the sample dissolved in sufficient $H_2O$ to make 100 cc) or, if the sample is insoluble in $H_2O$, 1 g of the sample and 10 cc of $H_2O$, to a 200 cc glass-stoppered measuring cylinder; add 1.2 cc of 12% HCl; mix; and add absolute alcohol to the 200 cc mark. Mix thoroly and set aside for several hours. If necessary, make up to volume, filter, transfer 100 cc of the filtrate to a Kjeldahl flask, evaporate the alcohol on a water bath, and determine N in the residue as directed under **II, 19, 22** or **24.**

### 52 CREATIN—OFFICIAL

Dissolve about 7 g of the sample in cold (20°) $NH_3$-free $H_2O$ in a 150 cc beaker, transfer the soln to a 250 cc volumetric flask, dilute to the mark, and mix thoroly. Transfer a 20 cc aliquot of this soln to a 50 cc volumetric flask and proceed as directed under **31.** Subtract from the combined creatinin value the equivalent of the pre-formed creatinin, **53,** and multiply the difference by 1.16 to convert into creatin. Express the result as percentage of creatin.

### 53 CREATININ—OFFICIAL

Measure about 5 cc of the soln used in **52** into a 500 cc volumetric flask, add 10 cc of 10% NaOH soln and 30 cc of saturated picric acid soln (1.2%), mix, and rotate for 30 seconds. Allow to stand exactly 4.5 min. and then dilute to the mark at once with $H_2O$. Shake thoroly and read the depth of color after standing. If the reading is less than 7 or more than 9.5 mm, repeat, calculating the quantity of soln necessary to obtain a reading of about 8 mm. Express the result as percentage of creatinin, making the calculations as indicated under **31.**

### 54 NITRATES (INCLUDING ALSO NITRITES)—TENTATIVE

Proceed as directed under **12** or **14.**

### 55 GLYCEROL[17]—TENTATIVE

Weigh 2 g of a solid or 5 g of a liquid preparation in a small lead dish or thin glass shell containing 20 g of ignited sand. Transfer the dish and its contents to a mortar containing more ignited sand and several grams of anhydrous $Na_2SO_4$ and mix thoroly. Transfer the mixture, including the dish, to a Soxhlet apparatus that has a piece of cotton placed in the side arm to prevent solid particles from being siphoned over. Extract the entire mass with redistilled anhydrous acetone for 10 hours. Distil the acetone from the extract, carefully removing the last trace by means of a vacuum pump. Take up the residue in $H_2O$, add 5 cc of 10% $AgNO_3$ soln, dilute to a volume of 100 cc, shake, allow to stand overnight, filter, and determine glycerol in an aliquot of the filtrate as directed under **XXXIII, 63,** beginning with "Add 1 cc of sulfuric acid." With solid meat and yeast extracts a blank of 0.5–1.0% is obtained in most cases.

### 56 SUGAR—TENTATIVE

Heat 20 g of the sample with about 200 cc of $H_2O$ on a steam bath until all soluble substances have gone into soln, cool, and proceed from this point as directed under **22.** Reducing sugars to the extent of 0.5% may be present as a natural constituent of meat extracts.

### 57 PRESERVATIVES—OFFICIAL

Proceed as directed under **XXXII.**

### 58 METALS—TENTATIVE

Proceed as directed under **XXIX.**

## GELATIN[18]—TENTATIVE

### 59 PREPARATION OF SAMPLE

In the case of ground gelatin mix thoroly. In the case of sheet gelatin break the sheets into small pieces by hand. Further comminution is unnecessary in either case.

### 60 MOISTURE

Use a flat-bottomed metal dish approximately 55 mm in diameter and provided with a tightly fitting slip-in cover. Heat dish and cover to constant weight at 100°, cool, and weigh. Add approximately 2 g of the sample, prepared as directed under **59**; cover loosely, and reweigh. Place the dish uncovered in a water-jacketed oven and dry for 6 hours at the temp. of boiling $H_2O$. Press the cover firmly in place, remove the dish from the oven, cool in a vacuum desiccator over $H_2SO_4$, and weigh. In releasing the vacuum admit the incoming air thru $H_2SO_4$. Report the loss in weight as moisture.

### 61 ASH

Ignite at low redness, preferably in a muffle, as directed under **XXVII, 8.**

### 62 TOTAL PHOSPHORUS

Treat the ash obtained under **61** with 2–3 cc of $HNO_3$ and evaporate to dryness on a steam bath. Repeat the $HNO_3$ treatment and evaporation, take up the residue in hot $H_2O$ containing a few drops of $HNO_3$, and proceed as directed under **II, 7.**

### 63 NITROGEN

Proceed as directed under **II, 19, 22** or **24,** using a weighed quantity (about 2 g) of the sample. In the Kjeldahl and Gunning methods digest with the $H_2SO_4$ for at least 4 hours after the mixture has become clear and in the Kjeldahl-Gunning-Arnold method for 2 hours after the mixture has become clear.

### 64 ARSENIC[19]

Heat 20 g of the sample with 75 cc of As-free HCl (1+3) in a covered vessel until all insoluble matter has flocculated and the gelatin is dissolved. Add an excess of Br water (about 20 cc); neutralize with $NH_4OH$; add 0.5 cc of 85% $H_3PO_4$, or 2 g of $Na_2HPO_4 . 12H_2O$), or 2 g of crystallized $NaNH_4HPO_4 . 4H_2O$; and allow to cool. Precipitate the arsenic acid along with the phosphoric acid by adding an excess (about 30–35 cc) of magnesia mixture [**II, 5 (c)**]. Allow to stand for about an hour, filter, wash the precipitate several times with $NH_4OH$ (1+15), drain well, and dissolve in As-free HCl (1+3) to 50 cc volume in a volumetric flask. Take a 25 cc aliquot, dilute to 40 cc with HCl (1+3), and proceed as directed under **XXIX, 4,** beginning with "add 5 cc of the KI reagent." Use HCl in preparing the standard stains and run a blank determination on the reagents used. Arsenic impurities, if present, are usually found in the phosphate added.

## COPPER AND ZINC

### 65 COPPER*

Hydrolyze 50 g of the sample with 150 cc of HCl (1+3), as directed under **64,** heating about 2 hours on a steam bath. To facilitate filtration and separation from

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* A method based upon the determination of copper and zinc in the ash will be found in the report of the Referee on Gelatin, Vol. **14,** No. 4 (1931).

Zn and Fe later, use the phosphoric acid or phosphate, and magnesia mixture, as directed under **64.** Precipitate with $H_2S$ in a slightly ammoniacal soln. Allow the precipitate to settle, filter, and wash with 5% $NH_4Cl$ soln saturated with $H_2S$. Dissolve off the Zn and Fe sulfides, $MgNH_4PO_4$, etc., in 75 cc of HCl (1+9) saturated with $H_2S$. Reserve the soln for the determination of Zn as directed under **66.**

Digest the filter and CuS with 4 cc of $H_2SO_4$ and sufficient $HNO_3$ until the residue fumes freely and remains perfectly colorless. Cool, and add 10 cc of $H_2O$ and a slight excess of Br water. Boil off the excess of Br, cool, and make alkaline with $NH_4OH$. Filter to remove any $Fe(OH)_3$ or other precipitate formed and wash the filter and precipitate with hot $H_2O$. Remove excess of $NH_3$ in filtrate by heating; then acidify with 1 cc of glacial acetic acid. Cool, add 1 g of KI, and titrate with 0.01 *N* $Na_2S_2O_3$ soln with or without starch indicator. 1 cc of 0.01 *N* $Na_2S_2O_3 = 0.63$ mg of Cu.

### 66 ZINC

Boil the Zn and Fe soln under **65** to expel all $H_2S$. Make the soln decidedly ammoniacal and then make it decidedly acid with HCOOH (1+1). Filter while hot to remove insoluble matter as alumina, etc., and then pass in rapidly a stream of $H_2S$ for 10 min. Warm the soln 15 min. on a steam bath; then remove and allow to stand for 30 min. before filtration. Filter, wash the precipitate of ZnS with 2% $NH_4CNS$ soln, dry, ignite at the highest temp. of a Bunsen burner, cool, and weigh the ZnO.

### 67 POLARISCOPIC CONSTANTS[20]

Prepare a soln of a concentration of 3 g per 100 cc by soaking 3 g of the sample in 40–50 cc of cold $H_2O$ for approximately 15 min., heating to complete soln at about 50° and diluting to a volume of 100 cc at 35°. Polarize at 35° in a 2 dm tube, using the Ventzke scale.

Cool a portion of the gelatin soln rapidly to 10–15° and pour into cold, dry 1 dm tubes before jelly has had time to form. Place the tube in a constant temp. bath at 15° for 18 hours to obtain equilibrium rotation, and then polarize at 15°. Double the reading to place it on the basis of a 2 dm tube.

In order to clarify cloudy samples before polarizing, digest the original 100 cc in a stoppered flask with approximately 10 g of lightly powdered $MgCO_3$ for at least 1 hour at 35–40° and filter thru a folded filter until clear, avoiding unnecessary evaporation.

The increase in levorotation (mutarotation) between 35° and 15° is an index of the jelly strength developed.

### SULFUR DIOXIDE

### 68 *Distillation Method*

Proceed as directed under **XXXII, 31.**

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# XXIX. METALS IN FOODS

## ARSENIC

### GUTZEIT METHOD—~~TENTATIVE~~ Official

#### REAGENTS

(a) *Nitric acid.*—As-free and containing not less than 67% $HNO_3$

(b) *Sulfuric acid.*—As-free and containing not less than 93% $H_2SO_4$.

(c) *Hydrochloric acid.*—As-free and containing not less than 35% HCl.

(d) *Stannous chloride soln.*—Dissolve 40 g of As-free $SnCl_2.2H_2O$ in HCl and make up to 100 cc with the same strength acid.

(e) *Hydrochloric acid-stannous chloride soln.*—Use in place of the HCl and $SnCl_2$ soln when a large number of determinations are to be run. Dissolve 45 g of $SnCl_2 . 2\,H_2O$ in a 6-pound bottle of HCl.

(f) *Zinc.*—Use 30-mesh, or finer, As-free granulated Zn, which needs no preliminary treatment, or cut As-free stick Zn into pieces 1 cm in length (preferably by means of a bolt clipper equipped with a guide and securely mounted). Activate the pieces by covering about 200 g of the broken Zn with HCl (1+3), to which has been added 2 cc of the $SnCl_2$, allowing the action to continue 15 min. Sort out distinctly inactive or overactive pieces and pour off the liquid. Wash the Zn free from acid with hot, distilled $H_2O$, or with clear running $H_2O$, and then rinse with hot, distilled $H_2O$. Select uniformly etched non-pitted Zn. To maintain a supply of uniform Zn when a large number of determinations are run, maintain a system of rotation, using the Zn from an original receptacle until the stock is exhausted and putting the used Zn into a second receptable. Discard nonuniform pieces and use the Zn from the second receptable after washing it with clear running $H_2O$. Repeat this procedure until the pieces are too small for further use.

(g) *Ammonium oxalate soln.*—Saturated.

(h) *Potassium iodide soln.*—Dissolve 15 g of KI in $H_2O$ and dilute to 100 cc.

(i) *Lead acetate cotton.*—No. 2 cotton dental rolls of the same apparent compactness, treated with 20% Pb-acetate soln.

(j) *Mercuric bromide paper.*—Cut heavy, cold-pressed, close-textured drafting paper, similar to Whatman No. 40, into strips exactly 2.5 mm wide and about 12 cm long. (This paper can be purchased in individual strips or in sheets of cut strips. If purchased already cut, examine the strips carefully to see that they have been cut from paper of the same weight and texture and are uniform in width. Uniformity of width and character of paper are of first importance; slight variations may cause considerable difference in the length of stain produced by the same quantity of As. The Hanford-Pratt or similar As strips have proved to be very satisfactory.) To sensitize, soak the strips for 1 hour in a 3–6 per cent soln of $HgBr_2$ in 95% alcohol, according to the quantity, character, and activity of the Zn used. If the strips are in sheets, cut off two sides before soaking and leave the strips attached at the ends. The length and density of stain for any given strips are controlled primarily by the Zn and secondarily by the strength of the $HgBr_2$. A 4% soln of $HgBr_2$ is satisfactory in most instances.) The sensitizing soln of $HgBr_2$ should also be used in a fresh condition—in no case after a precipitate begins to deposit. After sensitization remove the strips and dry the individual ones on glass rods and the groups by waving them in the air. (If desired, place the strips when nearly dry between clean sheets of paper and subject them to pressure long enough to take out any bends or curls.)

Avoid touching the sensitized strips with the hands except when necessary. When the individual strips are used, cut them off squarely about half an inch from one end and insert this end into the narrow tube. Compare stains only with a curve obtained from standard strips run at the same time and from the same strip-group. (Aging of the impregnated strips usually results in a markedly fainter and longer stain. The most desirable type of stain results from the use of strips that have not been impregnated longer than 2 days.)

(k) *Standard arsenic soln.*—Dissolve 1 g of $As_2O_3$ in 25 cc of 20% NaOH. Saturate the soln with $CO_2$ and dilute to 1 liter with recently boiled $H_2O$. 1 cc of this soln contains 1 mg of $As_2O_3$. Dilute 40 cc of this soln to 1 liter. Make 50 cc of the diluted soln to 1 liter and use to prepare the standard stains. 1 cc of the latter soln contains 0.002 mg of $As_2O_3$. A soln containing 0.001 mg $As_2O_3$ may also be prepared if desired. Prepare fresh dilute solns at frequent intervals.

### 2 APPARATUS

*Generators and absorption tubes.*—Use 2-oz., wide-mouthed bottles of uniform capacity and design as generators, and fit by means of a perforated stopper with a glass tube 1 cm in diameter and 6–7 cm long, with an additional constricted end to facilitate connection. Fit each tube with a 2 inch length of No. 2 cotton dental roll, which should come within 0.6–1.2 cm of the rubber stopper on the upper end. A convenient method to keep the cotton in all the tubes uniformly moist and compact is to immerse the lower end of the scrubber tube in the Pb-acetate soln, thus saturating the cotton to a depth of approximately 1.2 cm. Shake or blow out the excess. If the cotton has dried out thru disuse, remoisten it. Connect the tube by means of a rubber stopper with a narrow glass tube 2.6–2.7 mm in internal diameter and 10–12 cm long, containing the strip of $HgBr_2$ paper. (A 3 mm tube allows the strip to curl, which results in an uneven stain and a poor end point.) Clean and dry the tube before inserting the bromide paper. An ordinary pipe cleaner may be used.

*Water Bath.*—Use any constant temp. water bath. If no water bath is available, use any flat-bottomed container of suitable depth and capacity. (A deep water bath is suggested to insure uniform conditions during evolution and absorption of the As.)

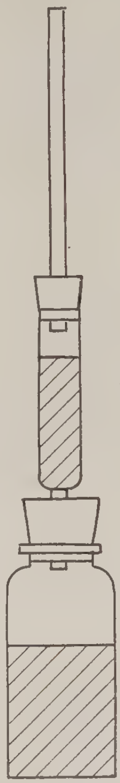

FIG. 31.

GENERATOR TO BE USED WITH THE GUTZEIT METHOD

### 3 PREPARATION OF SOLUTION

(a) *For fresh fruits* (*apples, pears or similar products*).—Weigh and peel 1–2 lbs of sample, choosing fruit that shows the largest amount of spray residue. (When 700 g of sample and 1/100th aliquot are employed, the strip reading in mg of $As_2O_3$ equals grains per pound of fruit. To obtain exactly 700 g the last fruit may be segmented from calyx to stem.) At the blossom and stem end make a deep, wide conical cut to include the flesh thought to be contaminated with arsenical compounds. Place the peelings in an 800 cc Pyrex Kjeldahl flask. ("Wet ashing" apparatus of Duriron is now available and may be used.) Add 60–75 cc of $HNO_3$, then add cautiously 20 cc of $H_2SO_4$. When the excessive foaming subsides, heat on an asbestos mat which has a 2-inch hole in the center. When the mixture turns brown or black, add *cautiously* a small quantity of $HNO_3$, heat, and as browning recurs repeat the addition of $HNO_3$. Repeat the heating and addition of $HNO_3$ until the organic matter is destroyed and $SO_3$ fumes are copiously evolved. The final soln should be water-white, or at most, a light straw color. Cool slightly and add 75 cc of $H_2O$ and 25 cc of the saturated soln of $NH_4$-oxalate to assist in expelling all traces of oxides of N from the soln. Evaporate again to fumes. Cool, and dilute with distilled $H_2O$ to 500 or 1000 cc in a volumetric flask.

(**b**) *For dried fruit products.*—Prepare the sample by alternately grinding and mixing 4–5 times in a food chopper. Place 70 g portions in 800 cc Kjeldahl flasks, and add at first 50 cc of $HNO_3$ and only 5 cc of $H_2SO_4$. Warm slightly until brown fumes commence to appear; then turn off the heat and allow to proceed spontaneously until the initial excessive foaming has subsided. (Heat may be cautiously applied when the digestion commences to proceed more quietly.) Add $HNO_3$ whenever browning of the soln appears. Shake the flask or turn frequently to prevent the sample from charring and caking upon the glass exposed to the flame. As the rate of digestion becomes slower, add more $H_2SO_4$ until a total of 20 cc has been reached. Continue the digestion as directed under (**a**), beginning with "Repeat the heating," then dilute the soln to 250 cc. When the above quantities and dilutions are used the strip reading on a 25 cc aliquot gives grains of $As_2O_3$ per pound of dried fruit direct.

(**c**) *For small fruits, vegetables, etc.*—Use 70–140 g of fruit and digest as described under (**b**).

(**d**) *For dry, finely divided food products.*—Use 5–50 g and digest as directed under (**b**). Dilute to definite volume (25–250 cc).

### 4 DETERMINATION

Place one or more 5–30 cc aliquots in the Gutzeit generator, gaging the aliquots so that they contain approximately 0.02 mg of $As_2O_3$. (An aliquot containing 0.02–0.025 mg of $As_2O_3$ is considered optimum for reading the stain. A certain degree of error results from reading either very long or very short stains. In the case of long stains, there is an increased intensity due to the relatively large quantity of As, which shortens the stain disproportionately. With short stains any error made in locating the end point is a greater proportion of the stain than is the case of the longer stain.) Add to the acid in the aliquot sufficient HCl or HCl-$SnCl_2$ soln to make a total volume of 5 cc [$H_2SO_4$ (93%) and HCl (35%) are considered equivalent to each other for this purpose.] Add 5 cc of the KI reagent and 4 drops of $SnCl_2$ soln unless the latter reagent has already been added in the form of HCl-$SnCl_2$ soln. From the standard As soln prepare standards corresponding to 0.010, 0.015, 0.020, and 0.025 mg of $As_2O_3$. Add $H_2SO_4$ and HCl in quantities equivalent to the quantities in the unknown. Mix, and allow to stand for at least 10 min. at a temp. of 20–25°. (It is not necessary to heat the generator containing the reagents or aliquots to 90° to effect reduction of arsenate to arsenite. Allowing the aliquot to stand at 20° for 10–15 min. effects the reduction more conveniently. Dilute with distilled $H_2O$ to 40 cc.

Prepare the generator as described under **2** and center a strip of $HgBr_2$ paper carefully in the narrow tube. According to the activity of the Zn, add to each of the standards and samples 10–15 g of activated stick Zn or 2–5 g of granulated Zn. Equalize as far as possible the area of Zn exposed in standard and sample. The surface area of granulated Zn is so large that moderate differences in weights of Zn have little influence on it.

Immerse the apparatus to within 1 inch of the top of the narrow tube in the water bath, which is kept at a constant temp. between 20 and 25°, and allow the evolution to proceed for $1\frac{1}{2}$ hours. Remove the strip and average the length of the stains on both sides in mm. Plot a graph of the standard strips on cross-sectioned paper, using the length in mm as ordinates and the mg of $As_2O_3$ as abscissa. (The preparation of a standard graph averages the errors of individual standards. Reading the strip from such a graph is considered more convenient and accurate than comparing the strips themselves.) Locate the length of the unknown strip on a standard graph and read off on the abscissa the quantity of As present. Report only to the third decimal as grains of $As_2O_3$ per pound. Take smaller or larger aliquots when the stain is longer or shorter than the highest or lowest standard, respectively.

Frequent blanks should be made. With reagents of suitable quality, blanks should not show more than 0.001 mg of $As_2O_3$.

## HEAVY METALS

### TREATMENT OF SAMPLE—TENTATIVE

### 5 *Destruction of Organic Material*

The method employed for the destruction of the organic matter in a foodstuff depends upon the percentage of moisture present and upon the nature of the metal under consideration. Finely divided foods containing much moisture or fatty matter should be dried first, then extracted with ether to facilitate the subsequent combustion. The weight of the sample to be taken should vary according to the probable amount of metal that is likely to be present. The result is usually reported as parts per million. One-millionth of a 50-g sample = 0.05 mg. This equivalent will serve as a guide for estimating the weight of sample needed for the most advantageous estimation of metals.

(**a**) *Ashing.*—Destroy the organic matter by direct ashing in the presence of $H_2SO_4$. For each 5 g of sample add 0.5 cc of $H_2SO_4$, ignite gently until the sample is well carbonized, then burn in a muffle at low redness until the organic matter is destroyed. The advantage of sulfate ashing is that the sulfates of the metallic bases are less liable to be driven off thru overheating.

(**b**) *Moist combustion.*—Weigh 50–100 g of the sample, depending upon the percentage of moisture and the relative ease with which the organic matter is oxidized, into an 800 cc Kjeldahl flask and add 50 cc of $HNO_3$ in small portions. Allow the mixture to stand overnight, then place the flask on asbestos over a low flame until the contents boil quietly. Add 10–15 cc of $H_2SO_4$ and heat until white fumes are generated. Cool somewhat, add 5–10 cc of $HNO_3$, and heat as before. Repeat the addition of $HNO_3$ until the soln remains clear after the $HNO_3$ is boiled off and fumes of $SO_3$ appear.

### TIN

### 6 *I. Gravimetric Method*[2]*—Tentative*

Add 200 cc of $H_2O$ to the digested sample and transfer to a 600 cc beaker. Rinse the Kjeldahl flask [see **5** (**b**)] with 3 portions of boiling $H_2O$, making a total volume of approximately 400 cc. Cool, and add $NH_4OH$ until just alkaline, then 5 cc of HCl or 5 cc of $H_2SO_4$ (1+3) for each 100 cc of soln. Place the beaker, covered, on a hot plate; heat to about 95°; and pass in a slow stream of $H_2S$ for an hour. Digest at 95° for an hour and allow to stand 1–2 hours longer. Or, dissolve the ash [**5**(**a**)] in HCl (1+4) cautiously, make up to approximately 400 cc and proceed as above.

Filter, and wash the precipitate of SnS alternately with 3 portions each of wash soln (100 cc of saturated $NH_4$-acetate soln, 50 cc of glacial acetic acid, and 850 cc of $H_2O$) and hot $H_2O$. Transfer the filter and precipitate to a 50 cc beaker, add 10–20 cc of $NH_4$-polysulfide, heat to boiling, and filter. Repeat the digestion with $NH_4$-polysulfide and the filtration twice, and then wash the filter with hot $H_2O$. Acidify the combined filtrate and washings with acetic acid (1+9), digest on a hot plate for an hour, allow to stand overnight, and filter thru a double 11 cm filter. Wash alternately with 2 portions each of the wash soln and hot $H_2O$ and dry thoroly in a weighed porcelain crucible. Ignite over a Bunsen flame, very gently at first to burn off filter paper and to convert the sulfide to oxide; then partly cover the crucible and heat strongly over a large Bunsen or Meker burner. (SnS must be roasted gently to the oxide, which may be heated strongly without loss by volatilization.) Weigh as $SnO_2$ and calculate to metallic tin, using the factor 0.7877.

### *II. Volumetric Method*[3]—*Tentative*

**7** REAGENTS

(a) *Air-free wash soln.*—Dissolve 20 g of $NaHCO_3$ in 2 liters of boiled $H_2O$ and add 40 cc of HCl. This soln should be freshly prepared.

(b) *Iodine.*—0.01 *N*. Standardize the soln frequently against (d), adding an asbestos mat and proceeding as described under **8**, omitting the precipitation with $H_2S$ and the boiling with HCl and $KClO_3$. The quantity of tin in the soln used for the standardization should equal approximately that contained in the sample under examination.

(c) *Sodium thiosulfate.*—0.01 *N*.

(d) *Standard tin soln.*—Dissolve 1 g of Sn in about 500 cc of HCl and dilute to 1 liter with $H_2O$. 1 cc contains 1 mg of Sn.

(e) *Sheet aluminum.*—About 30 gage, free from Sn.

(f) *Starch indicator.*—Prepare as directed under **VI, 3 (e)**.

**8** DETERMINATION

Proceed as directed under **6** to "Digest at 95° for an hour and allow to stand 1–2 hours longer."

Filter thru asbestos in a Gooch crucible having a detachable bottom, using suction. Wash the precipitate of SnS a few times and then transfer the detachable bottom and asbestos pad to a 300 cc Erlenmeyer flask. Remove all traces of the precipitate from the inside of the crucible by means of a jet of hot $H_2O$ and a rubber-tipped rod, using a minimum quantity of $H_2O$ for washing.

Add to the flask 100 cc of HCl and 0.5 g of $KClO_3$. Boil for approximately 15 min., making about 4 more additions of smaller quantities of the $KClO_3$ as Cl is boiled out of the soln. Wash the particles of $KClO_3$ down from the neck of the flask with $H_2O$ and finally boil to remove Cl. Then add about 1 g of the sheet Al to dispel the last traces of Cl.

Fit a 2-holed rubber stopper to the flask. Thru one of the holes pass a bulbed glass tube that reaches nearly to the surface of the liquid. Attach this tube to a large $CO_2$ generator thru a scrubber containing $H_2O$. The $CO_2$ passes out of the flask thru a short, bulbed tube inserted in the second hole of the rubber stopper and terminating slightly below it. Connect this second glass tube by means of a rubber tube with another glass tube, approximately 10 inches long, which is immersed in a cylinder of $H_2O$ to a depth of approximately 8 inches. This connection will act as a seal to restrain any strong flow of gas when not desired and to permit of a pressure in the flask.

Raise the delivery tube nearly out of the $H_2O$ seal, thus allowing a rapid flow of $CO_2$ for a few min. to dispel the air from the system. Then lower the delivery tube into the $H_2O$ seal, slightly raise the stopper, and quickly drop into the flask 1–2 g of Al foil, folded into a narrow bent strip to prevent breaking the flask. When the

Al has completely dissolved, raise the tube in the $H_2O$ seal, allowing the $CO_2$ to pass thru rapidly; place the flask upon a hot plate; and boil for a few min. Remove the flask from the heat and cool with tap or ice $H_2O$, continuing the flow of $CO_2$. Lower the delivery tube into the cylinder; disconnect the flask; and, with a glass plug, close the rubber tube thru which the $CO_2$ enters the flask. Wash the glass tubes, rubber stopper, and sides of the flask with the air-free wash soln; add the starch indicator; and titrate immediately with the 0.01 $N$ I soln.

If desired, the titration may be made by slightly raising the rubber stopper after cooling and adding an excess of the 0.01 $N$ I soln. Then disconnect the flask; wash the tubes, rubber stopper, and sides of flask with the air-free wash soln; and titrate the excess of I with the 0.01 $N$ $Na_2S_2O_3$.

## COPPER[4]

### *Volumetric Method—Tentative*

### 9 REAGENTS

(a) *Ammonium hydroxide.*

(b) *Sulfuric acid.*

(c) *Sodium thiosulfate soln.*—Saturated.

(d) *Ammonium sulfate soln.*—100 g made up to 1 liter.

(e) *Nitric acid.*—Sp. gr. 1.15. Prepare by diluting 20 cc of $HNO_3$ with 50 cc of $H_2O$.

(f) *Acetic acid.*—(1+1).

(g) *Potassium iodide.*

(h) *Standard copper soln.*—Dissolve 63.6 mg of pure metallic Cu in $HNO_3$ and evaporate to dryness on a steam bath. Add sufficient $H_2O$ and a few drops of acetic acid to dissolve the $Cu(NO_3)_2$ and again evaporate to dryness on a steam bath. Redissolve the $Cu(NO_3)_2$ as above and make up to 1 liter.

(i) *Sodium thiosulfate soln.*—0.01 $N$. Prepare a soln of $Na_2S_2O_3 . 5H_2O$ containing 2.5 g of pure crystals in 1 liter. Standardize daily against the standard Cu soln in the following manner: Place 20 cc of the standard Cu soln in a 100 cc Erlenmeyer flask and continue as directed under **10**, beginning with the words "add an excess of $NH_4OH$."

(j) *Starch indicator.*—Prepare daily as directed under **VI, 3** (e).

### 10 DETERMINATION

Neutralize the soln obtained by the wet digestion method or by dissolving the ashed sample in HCl, with $NH_4OH$. Add 5 cc of $H_2SO_4$, dilute the soln to 200 cc, and boil for 1 min. Then add cautiously 10 cc of a hot saturated soln of $Na_2S_2O_3$ and continue the boiling until the precipitate coagulates and the liquid becomes practically clear. (A few cc of the $(NH_4)_2SO_4$ soln may be added to hasten the coagulation.) Filter the precipitate and wash 6 times with hot $H_2O$. Reserve the filtrate for the determination of Zn, if necessary. Fold the precipitate within the filter paper, place in a small crucible, and ignite in an electric muffle at dull red heat. Dissolve the residue in 1 cc of $HNO_3$ and transfer to a 100 cc Erlenmeyer flask. Dry on the steam bath. Then dissolve the residue in 5 cc of $H_2O$ and again evaporate to dryness on the steam bath. Redissolve the $Cu(NO_3)_2$ in 20 cc of $H_2O$ and add an excess of $NH_4OH$. Boil the soln until it turns light brown; add acetic acid until it is acid to litmus paper and then 1 cc in excess. Again boil the soln for 1 min., cool to room temp., and add 2 g of KI dissolved in enough $H_2O$ to make the final soln 50 cc, including the 0.01$N$ $Na_2S_2O_3$ soln used in titrating. Titrate the free I immediately with 0.01 $N$ $Na_2S_2O_3$

soln until the end point is nearly reached, then add 2 cc of starch soln. Continue the titration dropwise to the end point. 1 cc of 0.01 *N* $Na_2S_2O_3$ = 0.6357 mg of Cu.

## ZINC

### *Gravimetric Method—Tentative*

### 11 REAGENTS

(**a**) *Ammonium hydroxide.*

(**b**) *Hydrochloric acid.*—(1+9) and (1+3).

(**c**) *Sodium or ammonium acetate soln.*—Dissolve 50 g of the salt in $H_2O$ and make up to 100 cc.

(**d**) *Hydrogen sulfide gas.*

(**e**) *Hydrogen sulfide wash $H_2O$.*

(**f**) *Bromine $H_2O$.*

(**g**) *Ammonium chloride.*

(**h**) *Ferric chloride soln.*—Dissolve 10 g of $FeCl_3$ in 100 cc of $H_2O$.

(**i**) *Methyl orange indicator.*—[**VI, 3 (f)**].

### 12 DETERMINATION

Proceed as directed under **10** to the sentence "Reserve the filtrate for the determination of Zn, if necessary." Boil the filtrate containing the Zn to expel $H_2S$ and to reduce the volume to about 250–300 cc; add a drop of methyl orange indicator and 5 g of $NH_4Cl$, and make alkaline with $NH_4OH$. Add HCl (1+9), dropwise, until the reaction is faintly acid; then add 10–15 cc of 50% Na- or $NH_4$-acetate soln; and pass in $H_2S$ for a few min. until precipitation is complete. Allow the precipitate to settle; filter; refilter, if necessary, until the filtrate is clear; and wash the precipitate twice with $H_2S$ water. Dissolve the precipitate on the filter with a little HCl (1+3), wash the filter with $H_2O$, boil the combined filtrate and washings to expel $H_2S$, cool, and add a distinct excess of Br water. Then add 5 g of $NH_4Cl$ and $NH_4OH$ until the color, caused by free Br, disappears. Add HCl (1+3), dropwise, until the Br color just reappears; then add 10–15 cc of Na- or $NH_4$-acetate soln (50% by weight) and 0.5 cc of $FeCl_3$ soln (10 g per 100 cc), or enough to precipitate all the phosphates. Boil until all the Fe is precipitated. Filter while hot and wash the precipitate with $H_2O$ containing a little Na-acetate. Pass $H_2S$ into the combined filtrate and washings until all the ZnS, which should be pure white, is precipitated; filter upon a weighed Gooch crucible previously heated to constant weight; and wash with $H_2S$ water containing a little $NH_4NO_3$. Dry the crucible and its contents in an oven, ignite at a bright red heat, cool, and weigh as ZnO. Calculate the weight of metallic Zn, using the factor 0.8034.

### 13 LEAD—TENTATIVE

Char the sample *without adding $H_2SO_4$*. Complete the ashing process in a muffle and take up the ash with HCl. Proceed with either of the methods for Pb described under **XV**.

### 14 FLUORINE—TENTATIVE

Decompose the sample by ashing in the presence of enough $Na_2CO_3$ to insure alkalinity and complete the ignition in a muffle at a temp. below discernible redness (not over 450°). Remove the dish from the muffle, allow to cool, powder the charred mass, and thoroly saturate with 5 cc of $Mg(NO_3)_2$ soln. Proceed as directed under **XV, 38.**

### 15 MANGANESE—TENTATIVE

Proceed according to either of the official methods for the determination of Mn given under **XII.**

## SELECTED REFERENCES

[1] U. S. Dept. Agr. Bur. Chem. Circ , **1912**; J. Soc. Chem. Ind., **26**, 1115 (1907) J. Assoc. Official Agr. Chem., **7**, 48 (1923).
[2] J. Assoc. Official Agr. Chem., **1**, 257 (1915).
[3] Original Communications, VIII Intern. Cong. Appl. Chem., **18**, 35 (1912).
[4] J. Assoc. Official Agr. Chem., **13**, 426 (1930).

# XXX. NUTS AND NUT PRODUCTS*

* See note at bottom of p. xv.

# XXXI. OILS, FATS, AND WAXES

### 1 PREPARATION OF SAMPLE—OFFICIAL

Melt solid fats and filter by means of a hot water funnel or similar apparatus. Make the different determinations on samples of this melted, homogeneous mass. Filter oils that are not clear. Keep oils and fats in a cool place and protected from light and air, otherwise they will soon become rancid. Weigh out at one time as many portions as are needed for the various determinations, using a small beaker or weighing buret.

## SPECIFIC GRAVITY (APPARENT)

### *At 25°/25°—Official*

### 2 STANDARDIZATION OF PYCNOMETER

Carefully clean the pycnometer by filling it with a saturated soln of $CrO_3$ in $H_2SO_4$ and allowing to stand for several hours. Empty the pycnometer and rinse thoroly with $H_2O$; then fill it with recently boiled $H_2O$ previously cooled to about 20° and place in a constant temp. bath at 25°. At the end of 30 min. adjust the level of the $H_2O$ to the proper point on the pycnometer and put the perforated cap or stopper in place; remove from the bath, wipe dry with a clean cloth or towel, allow to stand for 30 min., and weigh. Empty the pycnometer, rinse several times with alcohol and then with ether, allow it to become perfectly dry, remove ether vapor, and weigh. Ascertain the weight of contained $H_2O$ at 25° by subtracting the weight of the pycnometer from its weight when full.

### 3 DETERMINATION

Fill the clean, dry pycnometer with the oil previously cooled to about 20°, place in a constant temp. bath at 25° for 30 min., adjust the level of the oil to the proper point on the pycnometer, and put the cap or stopper in place; remove from the bath, wipe dry, and weigh as directed under **2**. Subtract the weight of the empty pycnometer from its weight when filled with oil and divide the difference by the weight of $H_2O$ at 25°, as determined under **2**. The quotient is the specific gravity at 25/25° (apparent).

### 4 TEMPERATURE CORRECTION FOR SPECIFIC GRAVITY OF OILS—OFFICIAL

If the specific gravity of the oil is determined at other than standard temp., the approximate specific gravity at 25° may be calculated by means of the following formula:

$G = G' + 0.0007\ (T - 25°)$, in which
$G$ = specific gravity at 25°;

$G'$ = specific gravity at $\frac{T°}{25°}$;

$T$ = temp. at which the specific gravity was determined; and
0.0007 = mean correction[1] for 1°.

### *At the Temperature of Boiling Water—Official*

### 5 STANDARDIZATION OF FLASKS

(a) Weigh a 25–30 cc specific gravity flask and fill with freshly boiled hot $H_2O$.

Place in a briskly boiling water bath for 30 min., replacing any evaporation from the flask by the addition of boiling $H_2O$. Then insert the stopper, previously heated to 100°, remove the flask, cool, and weigh.

(b) The following formula may be used for calculating the weight of $H_2O$ ($W^T$) which a given flask will hold at $T°$ (weighed in air with brass weights at the temp. of the room) from the weight of $H_2O$ ($W^t$) (weighed in air with brass weights at the temp. of the room) contained therein at $t°$:

$$W^T = W^t \frac{d^T}{d^t} [1+0.000026\ (T-t)], \text{ in which}$$

$d^T$ = the density of $H_2O$ at $T°$; and
$d^t$ = the density of $H_2O$ at $t°$.

## 6 DETERMINATION

Fill the dry flask with the dry, hot, freshly filtered fat, which should be entirely free from air bubbles, and keep in a water bath for 30 min. at the temp. of boiling $H_2O$. Insert the stopper, previously heated to 100°, cool, and weigh. Divide the weight of contained fat by the weight of contained $H_2O$ previously found to obtain the specific gravity.

The weight of $H_2O$ at boiling temp. must be determined under the barometric conditions prevailing at the time the determination is made.

## INDEX OF REFRACTION

### 7 *General Directions—Official*

Place the instrument in such a position that diffused daylight or any form of artificial light can readily be obtained for illumination. Circulate thru the prisms a stream of $H_2O$ of constant temp. Determine the index of refraction with any standard instrument, reading oils at 20° and fats at 40°. The readings of the Zeiss butyro-refractometer on fats may be reduced to standard temp. by the following formula[2]:

$$R = R' + 0.55\ (T' - T), \text{ in which}$$

$R$ = the reading reduced to temp. $T$;
$R'$ = the reading at $T'$;
$T'$ = the temp. at which reading $R'$ is made;
$T$ = the standard temp.; and
0.55 = correction in scale divisions for 1°.

With oils the factor 0.58 is substituted in the formula for 0.55, because they have a higher index of refraction. The readings of instruments that give the index of refraction directly can be reduced to standard temp. by substituting the factor 0.00038 for 0.55 in the formula. As the temp. rises the refractive index falls. The instrument used may be standardized with $H_2O$ at 20°, the theoretical refractive index of $H_2O$ at that temp. being 1.3330. Any correction found should be made on all readings. The index of refraction varies with the specific gravity and in the same direction. If the results appear abnormal, compare the specific refractive power[3] with the normal. Calculate the specific refractive power from the formula $\frac{N-1}{D}$, in which $N$ equals the refractive index and $D$ the specific gravity. According to Proctor,[4] the Lorenz formula, $\frac{N^2-1}{(N^2+2)\ D}$, gives much more satisfactory results than $\frac{N-1}{D}$.

### 8 *I. By Means of the Abbé Refractometer—Official*

To charge the instrument, open the double prism by means of the screw head and place a few drops of the sample on the prism or, if preferred, open the prisms slightly by turning the screw head and pour a few drops of the sample into the funnel-shaped aperture between the prisms. Then close the prisms firmly by tightening the screw head. Allow the instrument to stand for a few min. before the reading is made, so that the temp. of the sample and the instrument will be the same.

The method of measurement is based upon the observation of the position of the *border line of total reflection* in relation to the faces of a prism of flint glass. Bring this border line into the field of vision of the telescope by rotating the double prism by means of the alidade in the following manner: Hold the sector firmly, move the alidade backward or forward until the field of vision is divided into a light and a dark portion. The line dividing these portions is the "border line," and, as a rule, will not be a sharp line but a band of color. The colors are eliminated by rotating the screw head of the compensator until a sharp, colorless line is obtained. The border line should now be adjusted so that it falls on the point of intersection of the cross hairs. Read the refractive index of the substance directly on the scale of the sector. Check the correctness of the instrument as directed under **7**, or by means of the quartz plate that accompanies it, using monobromnaphthalene, and make the necessary correction in the reading.

### 9 *II. By Means of the Zeiss Butyro-Refractometer—Official*

Place 2 or 3 drops of the filtered fat on the surface of the lower prism. Close the prisms and adjust the mirror until it gives the sharpest reading. If the reading is indistinct after running $H_2O$ of a constant temp. thru the instrument for some time, the fat is unevenly distributed on the surfaces of the prism. As the index of refraction is greatly affected by temp., use care to keep the latter constant. Carefully adjust the instrument by means of the standard fluid that is supplied with it. Convert the degrees of the instrument into refractive indices from the table under **10**.

### 10 *Butyro-refractometer readings and indices of refraction*

| READING | INDEX OF REFRACTION | READING | INDEX OF REFRACTION | READING | INDEX OF REFRACTION | READING | INDEX OF REFRACTION |
|---|---|---|---|---|---|---|---|
| 40.0 | 1.4524 | 50.0 | 1.4593 | 60.0 | 1.4659 | 70.0 | 1.4723 |
| 40.5 | 1.4527 | 50.5 | 1.4596 | 60.5 | 1.4662 | 70.5 | 1.4726 |
| 41.0 | 1.4531 | 51.0 | 1.4600 | 61.0 | 1.4665 | 71.0 | 1.4729 |
| 41.5 | 1.4534 | 51.5 | 1.4603 | 61.5 | 1.4668 | 71.5 | 1.4732 |
| 42.0 | 1.4538 | 52.0 | 1.4607 | 62.0 | 1.4672 | 72.0 | 1.4735 |
| 42.5 | 1.4541 | 52.5 | 1.4610 | 62.5 | 1.4675 | 72.5 | 1.4738 |
| 43.0 | 1.4545 | 53.0 | 1.4613 | 63.0 | 1.4678 | 73.0 | 1.4741 |
| 43.5 | 1.4548 | 53.5 | 1.4616 | 63.5 | 1.4681 | 73.5 | 1.4744 |
| 44.0 | 1.4552 | 54.0 | 1.4619 | 64.0 | 1.4685 | 74.0 | 1.4747 |
| 44.5 | 1.4555 | 54.5 | 1.4623 | 64.5 | 1.4688 | 74.5 | 1.4750 |
| 45.0 | 1.4558 | 55.0 | 1.4626 | 65.0 | 1.4691 | 75.0 | 1.4753 |
| 45.5 | 1.4562 | 55.5 | 1.4629 | 65.5 | 1.4694 | 75.5 | 1.4756 |
| 46.0 | 1.4565 | 56.0 | 1.4633 | 66.0 | 1.4697 | 76.0 | 1.4759 |
| 46.5 | 1.4569 | 56.5 | 1.4636 | 66.5 | 1.4700 | 76.5 | 1.4762 |
| 47.0 | 1.4572 | 57.0 | 1.4639 | 67.0 | 1.4704 | 77.0 | 1.4765 |
| 47.5 | 1.4576 | 57.5 | 1.4642 | 67.5 | 1.4707 | 77.5 | 1.4768 |
| 48.0 | 1.4579 | 58.0 | 1.4646 | 68.0 | 1.4710 | 78.0 | 1.4771 |
| 48.5 | 1.4583 | 58.5 | 1.4649 | 68.5 | 1.4713 | 78.5 | 1.4774 |
| 49.0 | 1.4586 | 59.0 | 1.4652 | 69.0 | 1.4717 | 79.0 | 1.4777 |
| 49.5 | 1.4590 | 59.5 | 1.4656 | 69.5 | 1.4720 | 79.5 | 1.4780 |

## MELTING POINT OF FATS AND FATTY ACIDS

### *Wiley Method—Official*

11 REAGENT

*Alcohol-water mixture.*—The specific gravity should be the same as that of the fat to be examined. Prepare by boiling, separately, $H_2O$ and 95% alcohol for 10 min. to remove the gases that may be held in soln. While still hot pour the $H_2O$ into a test tube until it is almost half full. Nearly fill the test tube with the hot alcohol, pouring it down the side of the inclined tube to avoid too much mixing. If the alcohol is added after the $H_2O$ has cooled, air bubbles will make the mixture unfit for use.

12 DETERMINATION

Allow the melted and filtered fat to fall a distance of 15–20 cm from a dropping tube upon a piece of ice or upon the surface of cold Hg. The disks thus formed should be 1–1.5 cm in diameter and should weigh about 200 mg. Remove the disks when solid, and allow to stand 2–3 hours in order to obtain the normal melting point.

Place a 30×3.5 cm test tube, containing the alcohol-water mixture, in a tall 35×10 cm beaker containing ice and $H_2O$, and leave until the mixture is cold. Then drop a disk of fat into the tube. It will sink immediately to a point where the density of the alcohol-water mixture is exactly equivalent to its own. Lower an accurate thermometer, which can be read to 0.1°, into the test tube until the bulb is just above the disk. In order to secure an even temp. in all parts of the alcohol-water mixture around the disk, stir gently with the thermometer. Slowly heat the $H_2O$ in the beaker, constantly stirring it by means of an air blast or other suitable device.

When the temp. of the alcohol-water mixture rises to about 6° below the melting point of the fat, the disk of fat begins to shrivel and gradually rolls up into an irregular mass. Lower the thermometer until the fat particle is even with the center of the bulb. Rotate the thermometer bulb gently and so regulate the heat that about 10 min. is required for the last 2° increase in temp. As soon as the fat mass becomes spherical, read the thermometer. Remove the tube from the bath and again cool. Place in the bath a second tube containing the alcohol-water mixture. The test tube is of sufficiently low temp. to cool the bath to the desired point. After the first or preliminary determination, regulate the temp. of the bath so as to obtain a maximum of about 1.5° above the melting point of the fat under examination.

If the edge of the disk touches the sides of the tube, make a new determination. Run triplicate determinations. The second and third results should agree closely.

### 13 *Capillary Tube Method*[5]*—Official*

Draw the melted fat or fatty acids into a thin-walled capillary tube. Use a column of fat 1–2 cm long, according to the length of the thermometer bulb. Seal one end of the tube and cool on ice 12–15 hours. Attach the capillary tube to the bulb of an accurate thermometer graduated to 0.2°; immerse in a large test tube of $H_2O$ surrounded by a beaker of $H_2O$, and heat very slowly. An apparatus similar to that indicated in Fig. 32 may be used. Take as the melting point the temp. at which the substance becomes transparent.

## TITER TEST

### *Alcoholic or Aqueous Sodium Hydroxide Method—Official*

14 APPARATUS

*Standard thermometer.*—Use a thermometer having a zero mark, 0.1° graduations between 10° and 60°, and auxiliary reservoirs at the upper end and between the 0°

and 10° marks. The cavity of the capillary tube between the 0° and the 10° marks must be at least 1 cm below the 10° mark, which must be 3–4 cm above the bulb, the total length of the thermometer being about 38 cm. The bulb should be about 3 cm long and 6 mm in diameter, with scale etched on, and the graduations should be clear cut and distinct. The thermometer should be made of the best thermometric glass and thoroly annealed, so that scale errors will not increase after continued heating.

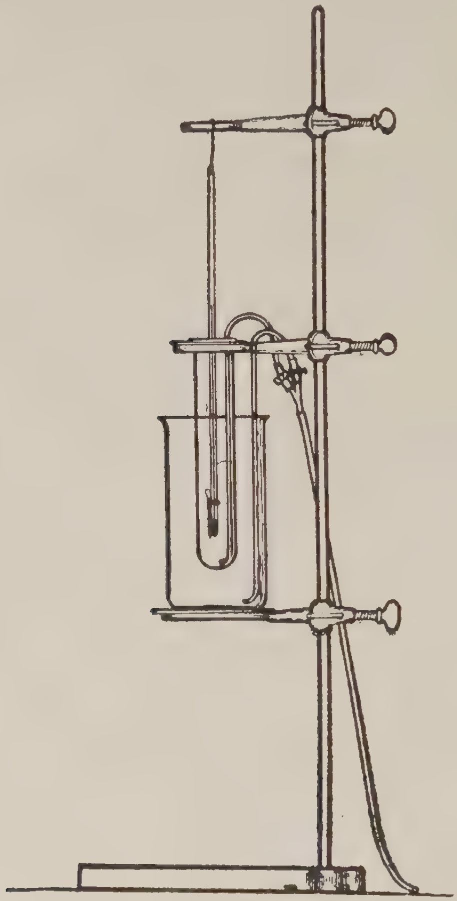

FIG. 32.—APPARATUS FOR THE DETERMINATION OF THE MELTING-POINT

### 15 DETERMINATION

Saponify 75 g of the sample in a metal dish with 60 cc of 30% NaOH soln (36° Baumé) and 75 cc of 95% alcohol or 120 cc of $H_2O$. Evaporate to dryness over a very low flame or on an iron or asbestos plate, stirring constantly. Dissolve the dry soap in 1 liter of boiling $H_2O$, and if alcohol has been used boil for 40 min. to remove it, adding sufficient $H_2O$ to replace that lost in boiling. Liberate the fatty acids by adding 100 cc of $H_2SO_4$ (1+3) and boil until they form a clear, transparent layer. Wash the fatty acids with boiling $H_2O$ until free from $H_2SO_4$, collect in a small beaker, and place on a steam bath until the $H_2O$ has settled and the fatty acids are clear. Decant into a dry beaker, filter while hot, and dry 20 min. at 100°. When dried, cool the fatty acids to 15–20° above the expected titer and transfer to the titer tube, 25 by 100 mm (1 by 4 inches) and made of glass about 1 mm in thickness. Place in a 16 ounce wide-mouthed bottle of clear glass, 70 by 150 mm (2.8 by 6 inches), fitted with a perforated cork so as to hold the tube rigidly when in position. Suspend the standard thermometer so that it can be used as a stirrer and stir the mass slowly until the Hg remains stationary for 30 seconds. Then allow the thermometer to hang quietly, with the bulb in the center of the mass, and observe the rise of the Hg column. The highest point to which it rises is regarded as the titer of the fatty acids.

Test the fatty acids for complete saponification as follows: Place 3 cc in a test tube and add 15 cc of 95% alcohol. Bring the mixture to a boil and add an equal volume of $NH_4OH$ (1+2). A clear soln should result. Make the titer at about 20° for all fats having a titer above 30°, and at 10° below the titer for all other fats.

### 16 *Glycerol-Potassium Hydroxide Method—Official*

Heat 75 cc of glycerol KOH soln (25 g of KOH in 100 cc of U. S. P. glycerol) to 150° in an 800 cc beaker and add 50 cc of the oil or melted fat, previously filtered if necessary to remove foreign substances. Saponification often takes place almost immediately, but heating and frequent stirring should be continued for 15 min. Do not use a temp. much above 150°. When the saponification is complete, as indicated by the perfectly homogeneous soln, pour the soap into an 800 cc casserole containing about 500 cc of nearly boiling $H_2O$; add carefully 50 cc of $H_2SO_4$ (1+3); and heat the soln, with frequent stirring, until the layer of fatty acids separates out perfectly clear. Transfer the fatty acids to a tall separatory funnel, wash 3 or 4 times with boiling $H_2O$ to remove all mineral acids, draw the fatty acids off into a small beaker, and allow to stand on a steam bath until the $H_2O$ has settled out and the acids are clear. Filter into a dry beaker and heat to 150° on a thin asbestos plate, stirring continually with the thermometer, transfer to a titer tube, fill it to within 2.5 cm of the top, and take the titer as directed under **15**.

## IODINE ABSORPTION NUMBER

(All reports should specify the method used)

### *Hanus Method—Official*

### 17 REAGENTS

(a) *Hanus iodine soln.*—Dissolve 13.2 g of pure I in 1 liter of glacial acetic acid (99.5%) that shows no reduction with dichromate and $H_2SO_4$. Add enough Br to double the halogen content as determined by titration (about 3 cc). The I may be dissolved by heating, but the soln should be cold when the Br is added.

A convenient procedure for preparing the Hanus I soln is as follows: Measure 825 cc of acetic acid that has shown no reduction by the dichromate test and dissolve in it with the aid of heat 13.615 g of I. Cool and titrate 25 cc of this soln with the 0.1 *N* $Na_2S_2O_3$. Measure another portion of 200 cc of the acetic acid and add 3 cc of Br. To 5 cc of this soln add 10 cc of the 15% KI soln, and titrate with the 0.1 *N* $Na_2S_2O_3$. Calculate the quantity of the Br soln required exactly to double the halogen content of the remaining 800 cc of I soln as follows:

$A = \frac{B}{C}$, in which

$A$ = cc of Br soln required;
$B$ = 800 × the thiosulfate equivalent of 1 cc of I soln; and
$C$ = the thiosulfate equivalent of 1 cc of Br soln.

Example: 136.15 g of I is dissolved in 8250 cc of acetic acid, and 30 cc of Br is dissolved in 2000 cc of acetic acid. Titrating 50 cc of the I soln against the standard thiosulfate shows that 1 cc of the I soln = 1.1 cc of the thiosulfate (0.0165 g of I). Titrating 5 cc of the Br soln shows that 1 cc of the Br soln = 4.6 cc of the thiosulfate. Then the quantity of Br soln required to double the halogen content of the remaining 8200 cc of I soln $= \frac{8200 \times 1.1}{4.6}$, or 1961 cc. Upon mixing the two solns in this proportion, there is obtained a total volume of 10161 cc, containing 135.3 g of I. In order to reduce this soln to the proper strength (13.2 g of I per liter), $10.161 \times 13.2 = 134.1$; $135.3 - 134.1 = 1.2$ g of I present in excess, or $\frac{1.2 \times 1000}{13.2} = 91$ cc of acetic acid, which must be added.

(b) *Sodium thiosulfate soln.*—0.1 *N*. Prepare a soln containing 24.82 g of $Na_2S_2O_3 \cdot 5H_2O$ in freshly boiled and cooled $H_2O$ and dilute to 1 liter. Standardize this soln as follows: Place in a glass-stoppered flask 20 cc of the 0.1 *N* $K_2Cr_2O_7$ and 10 cc of the 15% KI soln. Add 5 cc of HCl. Dilute with 100 cc of freshly boiled and cooled $H_2O$ and allow the 0.1 *N* $Na_2S_2O_3$ to flow slowly into the flask until the yellow color of the liquid has almost disappeared; add a few drops of the starch indicator; and with constant shaking, continue to add the 0.1 *N* $Na_2S_2O_3$ until the blue color just disappears.

(c) *Starch indicator.*—Prepare as directed under **VI, 3** (e).

(d) *Potassium iodide soln.*—Dissolve 150 g of KI in $H_2O$ and dilute to 1 liter.

(e) *Potassium dichromate.*—0.1 *N*. Dissolve 4.903 g of $K_2Cr_2O_7$ in $H_2O$ and dilute to 1 liter. The strength of this soln should be checked against pure Fe.

## 18 DETERMINATION

Weigh about 0.500 g of fat, or 0.250 g of oil (0.100–0.200 g in the case of drying oils that have a very high absorbent power), into a 500 cc glass-stoppered flask or bottle. Dissolve the fat, or oil, in 10 cc of $CHCl_3$. Add 25 cc of the Hanus I soln and allow to stand for 30 min., shaking occasionally. (This time must be adhered to closely in order to obtain accurate results. The excess of I should be at least 60% of the quantity added.) Add 10 cc of the 15% KI soln, shake thoroly, and then add 100 cc of freshly boiled and cooled $H_2O$, washing down any free I that may be found on the stopper. Titrate the I with the 0.1 *N* $Na_2S_2O_3$, adding it gradually, with constant shaking, until the yellow color of the soln has almost disappeared. Add a few drops of the starch indicator and continue the titration until the blue color has entirely disappeared. Toward the end of the titration, stopper the bottle and shake violently, so that any I remaining in soln in the $CHCl_3$ may be taken up by the KI soln. Conduct two blank determinations along with that on the sample. The number of cc of the 0.1 *N* $Na_2S_2O_3$ required by the blank less the quantity used in the determination gives the thiosulfate equivalent of the I absorbed by the fat or oil. Calculate the percentage by weight of I absorbed and report as the I number (Hanus method).

### *Wijs Method—Official*

**19** REAGENTS

*Wijs iodine soln.*—Dissolve 13 g of resublimed I in 1 liter of glacial acetic acid (99.5%) and pass in washed and dried Cl gas until the original thiosulfate titration of the soln is not quite doubled. Use not more than a slight excess of I and no excess of Cl. Preserve in a glass-stoppered amber bottle sealed with paraffin until ready for use. Mark, on the bottle or bottles, the date on which the soln is prepared and do not use Wijs soln that is more than 30 days old. Because of its unstable character $ICl_3$ should not be used for preparation of the I soln.[6]

The other reagents and solns used are described under **17.**

**20** DETERMINATION

Weigh 0.10–0.50 g (depending on the I number) of the melted and filtered sample into a clean, dry, 16 ounce, glass-stoppered bottle containing 15–20 cc of $CCl_4$ or $CHCl_3$. With a pipet add 25 cc of the I soln, allowing the pipet to drain for a definite time. The excess of I should be from 50–60% of the quantity added, that is, from 100–150% of the quantity absorbed. Moisten the stopper with the 15% KI soln to prevent loss of I or Cl but guard against the use of a quantity sufficient to run down inside the bottle. Let the bottle stand in a dark place for 30 min. at a uniform temp. At the end of that time add 20 cc of the 15% KI soln and 100 cc of recently boiled and cooled $H_2O$. Titrate the I with the 0.1 $N$ $Na_2S_2O_3$ soln, adding the latter gradually and with constant shaking until the yellow color of the soln has almost disappeared. Add a few drops of the starch indicator and continue the titration until the blue color has entirely disappeared. Toward the end of the reaction stopper the bottle and shake violently so that any I remaining in soln in the $CCl_4$ or $CHCl_3$ may be taken up by the KI soln. Conduct two determinations on blanks, run in the same manner as the sample, but do not use any fat. Slight variations in temp. affect quite appreciably the titer of the I soln as acetic acid has a high coefficient of expansion. It is essential, therefore, that the blanks and determinations on the sample be made at the same time. The number of cc of standard thiosulfate soln required by the blank less the quantity used in the determination gives the thiosulfate equivalent of the I absorbed by the sample taken. Calculate the percentage by weight of I absorbed and report as the I number (Wijs method).

## SAPONIFICATION NUMBER (KOETTSTORFER NUMBER)—OFFICIAL

**21** REAGENTS

(a) *Hydrochloric acid.*—0.5 $N$. Prepare as directed under **II, 17 (a).**

(b) *Alcoholic potassium hydroxide soln.*—Dissolve 40 g of the purest KOH in 1 liter of 95% alcohol, which has been previously redistilled from KOH over which it has been standing for some time, or with which it has been boiled for some time, using a reflux condenser. The soln must be clear and the KOH free from carbonates.

**22** DETERMINATION

Weigh accurately about 5 g of the filtered sample into a 250–300 cc Erlenmeyer flask. Pipet 50 cc of the alcoholic KOH soln into the flask, allowing the pipet to drain for a definite time. Connect the flask with an air condenser and boil until the fat is completely saponified (about 30 min.). Cool, and titrate with the 0.5 $N$ HCl, using phenolphthalein indicator. Conduct a blank determination along with that on the sample, using the same pipet for measuring the KOH soln and draining for the same length of time. Subtract the number of cc of 0.5 $N$ HCl obtained in the determination on the sample from the number obtained on the blank to obtain the cc of 0.5 *N* HCl equivalent to the KOH used in the saponification of the sample taken. Calculate and report as Koettstorfer number (mg of KOH required to saponify 1 g of fat).

### 23 SOLUBLE ACIDS—OFFICIAL

Place the flask used under **22** and its contents on a water bath and evaporate the alcohol. Add that quantity of 0.5 *N* HCl which is equivalent to the quantity of KOH used for the saponification of the sample under **22** and 1 cc more (quantity of 0.5 *N* HCl to be added = titration for blank − titration for sample + 1 cc), and place the flask on the steam bath until the separated fatty acids form a clear layer on the upper surface of the liquid. Fill the flask to the neck with hot $H_2O$ and cool the contents in ice $H_2O$ until the cake of fatty acids is thoroly hardened. Pour the liquid contents of the flask thru a filter into a liter flask, refill the flask with hot $H_2O$, and set on the steam bath until the fatty acids collect at the surface. Cool by immersing in ice $H_2O$, and again filter the liquid into the liter flask. Repeat this treatment with hot $H_2O$ 3 times, cooling and collecting the washings in the liter flask after each treatment. Titrate the combined washings with 0.1 *N* alkali, using phenolphthalein indicator. Subtract 5 (corresponding to the excess of 1 cc of 0.5 *N* acid) from the number of cc of 0.1 *N* alkali used and multiply by 0.0088 to obtain the weight of soluble acids as butyric acid. Calculate the percentage of soluble acids.

### 24 INSOLUBLE ACIDS (HEHNER NUMBER)—OFFICIAL

Allow the flask containing the cake of insoluble fatty acids from **23** and the paper thru which the soluble fatty acids have been filtered to drain and dry for 12 hours. Transfer the cake, together with as much of the fatty acids as can be removed from the filter paper, to a weighed, wide-mouthed beaker flask. Then place the funnel containing the filter in the neck of the flask and wash the paper thoroly with hot absolute alcohol. Remove the funnel, evaporate the alcohol, dry for 2 hours at 100°, cool in a desiccator, and weigh. Again dry for 2 hours, cool, and weigh. If there is any considerable decrease in weight, re-heat for 2 hours, cool, and weigh again. Calculate the percentage of insoluble fatty acids.

## SOLUBLE VOLATILE ACIDS (REICHERT-MEISSL AND POLENSKE VALUES)[7]—OFFICIAL

### 25 REAGENTS

(a) *Sodium hydroxide soln* (1+1).—Protect the soln from contact with $CO_2$. Allow the soln to settle and use only the clear liquid.

(b) *Sulfuric acid.*—Add 200 cc of concentrated acid to 800 cc of $H_2O$.

(c) *Sodium or potassium hydroxide soln.*—Standard 0.1 *N*.

(d) *Indicator.*—1 g of phenolphthalein in 100 cc of 95% (by volume) alcohol.

(e) *Pumice stone.*—Heat small pieces to a white heat, plunge into $H_2O$, and keep there until used.

(f) *Glycerol soda soln.*—Add 20 cc of the 1+1 NaOH soln to 180 cc of pure concentrated glycerol.

### 26 DETERMINATION

Weigh accurately 5 g of the sample to be tested into a clean, dry, 300 cc flask; add 20 cc of the glycerol-soda soln and heat over a flame or asbestos plate until complete saponification occurs, as shown by the mixture becoming perfectly clear. If foaming occurs, shake the flask gently. Add 135 cc of recently boiled $H_2O$, drop by drop at first to prevent foaming, then add 6 cc of the dilute $H_2SO_4$ and a few fragments of pumice stone. Distil without previously melting the fatty acids, using an apparatus of the approximate dimensions illustrated in the diagram (Fig. 33). Rest the flask on a piece of asbestos board having a hole 5 cm in diameter in the center, and so regulate the flame as to collect 110 cc of the distillate in as near 30 min. as possible and to allow the distillate to drip into the receiving flask at a temp. not higher than 18–20°.

When the distillation is complete, substitute for the receiving flask a 25 cc cylinder to collect any drops that may fall after the flame has been removed. Mix without violent shaking, immerse the flask containing the distillate almost completely in $H_2O$ at 15° for 15 min., filter the 110 cc of distillate thru a dry filter paper 9 cm in diameter, and titrate 100 cc with the standard NaOH soln, using phenolphthalein as an indicator. The pink color should remain unchanged for 2 or 3 min. The Reichert-Meissl value is the number of cc of 0.1 *N* NaOH soln used times 1.1., after this result is corrected for the figure obtained in a blank determination.

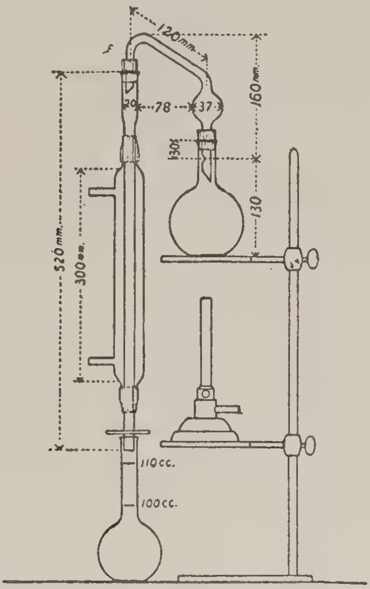

FIG. 33.—APPARATUS FOR THE DETERMINATION OF THE POLENSKE NUMBER

Remove the remainder of the soluble acids from the insoluble acids upon the filter paper by washing with 3 successive 15 cc portions of $H_2O$, previously passed thru the condenser, the 25 cc cylinder, and the 110 cc receiving flask. Then dissolve the insoluble acids by passing successive 15 cc portions of neutral alcohol, 95% by volume, thru the filter paper, each portion having previously passed thru the condenser, the 25 cc cylinder, and the 110 cc receiving flask. Titrate the combined alcoholic washings with the standard NaOH soln, using phenolphthalein as indicator. The Polenske value equals the number of cc of alkali soln required for the titration.

NOTE.—Unless these directions are followed in every detail as described, satisfactory results cannot be obtained.

### 27 *Leffman and Beam Method—Official**

### 28 KIRSCHNER VALUE[8]—OFFICIAL

#### REAGENTS

(a) *Barium hydroxide soln or sodium or potassium hydroxide soln.*—0.1 *N* in either case.

(b) *Silver sulfate.*

(c) *Alcoholic soln of phenolphthalein.*—1 g in 100 cc of 95% (by volume) alcohol.

(d) *Sulfuric acid.*—Add 25 cc of acid to 1000 cc of $H_2O$.

(e) *Pumice stone.*—Heat small pieces to a white heat, plunge into $H_2O$, and keep there until used.

#### 29 DETERMINATION

To 100 cc of the Reichert-Meissl distillate, in a 200 cc Erlenmeyer flask, add 6 drops of phenolphthalein soln and titrate to a very faint pink with a 0.1 *N* $Ba(OH)_2$ soln. Add 0.3 g of finely powdered $AgSO_4$. During the next hour shake the mixture frequently, then filter and transfer 100 cc of the filtrate into a 300 cc flask. Add 10 cc of the diluted $H_2SO_4$, 35 cc of $H_2O$, and a piece of Al wire or several small pieces of pumice stone. Distil 110 cc in about 20 min., using the Polenske apparatus, **26.** Titrate 100 cc of the distillate with 0.1 *N* $Ba(OH)_2$ soln, make a blank determination, and after correcting the number of cc of alkali soln used, calculate the Kirschner value according to the following formula: $K=\frac{A\times 121\ (100+B)}{10{,}000}$, where $A$ = the corrected Kirschner titration and $B$ = the number of cc of standard alkali soln to neutralize the 100 cc Reichert-Meissl distillate.

Butter fat gives Kirschner values from 19 to 26; coconut oil gives an average of 1.9 and palm kernel oil 1.0; whereas the majority of other fats and oils give values from 0.1 to 0.2.

## SATURATED AND UNSATURATED FATTY ACIDS

### LEAD SALT-ETHER METHOD[9]—OFFICIAL

#### 30 REAGENTS

(a) *Lead acetate soln.*—1000 cc of a 20% soln.

(b) *Potassium hydroxide soln.*—(1) Dissolve 50 g of KOH in 50 cc of $H_2O$; (2) dissolve 30 g of KOH in 200 cc of $H_2O$.

(c) *Acetic acid soln.*—Dissolve 25 cc of glacial acetic acid in 50 cc of $H_2O$.

(d) *Alcohol.*—95% by volume.

(e) *Ether.*—U. S. P. grade.

#### 31 DETERMINATION

Weigh accurately 10 or 20 g of the sample into a 200 cc Erlenmeyer flask. Add 30 cc of alcohol and 8 cc of the concentrated KOH soln [(b) (1)]. Mix thoroly and heat on the steam bath for about 30 min. Add a slight excess of acetic acid, using phenolphthalein as an indicator, and then add a sufficient quantity of the dilute KOH soln while rotating the flask to produce a distinct pink color. Heat to boiling in a liter flask 60 cc (120 cc for 20 g of sample) of Pb-acetate soln and the same quantity of $H_2O$. Add the neutralized soap soln cautiously to avoid any loss, rinsing the saponification flask with 5 cc of alcohol, then with small volumes of hot $H_2O$. Boil the mixture gently for about 5 min., shake thoroly, and cool under running $H_2O$, rotating the flask to cause all the precipitated lead soaps to adhere to the sides and bottom of the flask. When the mixture is cold pour off the aqueous soln into a large beaker in order to examine the soln for particles of Pb soap. (Usually the soln is slightly turbid owing to some basic Pb-acetate, and no particles or globules of Pb soap are seen.) Wash the flask and Pb soap twice with cold $H_2O$ and allow the flask to drain for 10 min. Remove the last drops of $H_2O$ by means of a thin roll of filter paper held by forceps, being careful to press the paper only lightly against the precipitate. Add about 120 cc of ether and shake by rotating the flask for about 5 min.

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* See note 7, p. xvii.

Connect the flask with a reflux condenser and boil the contents gently until the Pb soap is completely disintegrated or dissolved. Remove the flask and rinse down the sides with sufficient ether to make the final volume about 150 cc. Invert a close fitting beaker over the neck of the flask and place in an icebox for at least 15 hours. Place a 7 cm ordinary filter paper in a Büchner funnel of 7.5 cm diameter, turn on full suction, and fit a hardened filter paper cut to 8 cm in diameter as snugly as possible to the sides of the funnel. Decant the ether soln from the separated Pb soaps, using only sufficient suction to draw the ether thru the filter. (Too much suction causes the ether to evaporate so rapidly that the filter may become clogged with the separated unsaturated acids, Pb soaps, or ice.)

Transfer the precipitate to the filter by rinsing the flask with small portions of ether. During filtration keep the funnel covered as much of the time as possible to prevent the evaporation of the ether. If at any time filtration proceeds so fast as to cause the mass of Pb soap to crack, close the cracks by pressing with a small spoon or spatula; otherwise the precipitate cannot be properly washed. Rinse the spoon free from the precipitate and wash the precipitate 8 or 10 times with ether, finally allowing the suction to continue until the precipitate cracks into numerous pieces. Without delay, separate with a spoon as much of the precipitate as possible and transfer it without loss to a 500 cc separatory funnel containing about 50 cc of ether, washing off any precipitate adhering to the spoon and neck of the separatory funnel with ether. Transfer the filter paper to the liter flask. Shake the contents of the separatory funnel thoroly to disintegrate the lumps of lead salt and allow to stand for about 20 min. Add 20 cc of HCl previously diluted with 10 cc of $H_2O$ and shake thoroly for 2 min. to decompose all the Pb soap. Add 5–10 cc of HCl (2+1) to the liter flask containing the filter paper, shake thoroly to decompose any precipitate adhering to the flask and filter; then wash into the separatory funnel with small alternate portions of ether and $H_2O$ until all the fatty acids and $PbCl_2$ are removed from the flask. Again shake the contents of the separatory funnel with a rotary motion and allow to stand for 10 min. Withdraw the lower aqueous soln slowly, taking precautions not to remove any emulsion or undecomposed Pb soap. When Pb soap is present (shown in the form of lumps that float on top of the aqueous soln), add 10 cc of HCl and shake again; then add about 20 cc of $H_2O$, shake, and allow the mixture to stand until the layers have separated. Withdraw the aqueous soln and wash the ether with successive 25 cc portions of $H_2O$ until the washings are free from HCl. Dehydrate the ether with about 2 g of anhydrous $Na_2SO_4$ and transfer the soln to a weighed 300 cc Erlenmeyer flask. Rinse the separatory funnel and $Na_2SO_4$ with several small portions of ether to remove all the fatty acids, taking care not to allow any of the $Na_2SO_4$ to fall into the weighed flask. Distil the ether, avoiding any loss of the fatty acids, and heat in an oven at about 110° until the weight is constant. Obtain the weight of the saturated acids and save them for later investigation.

Transfer the ether soln of the soluble Pb soaps to a 500 cc or a 1000 cc separatory funnel, rinsing the Büchner funnel and filter flask with small quantities of ether. Add a mixture of 30 cc of HCl and 75 cc of $H_2O$ and shake with a rotary motion for 2 min. After allowing the mixture to stand for 10 min., slowly withdraw the aqueous soln into a beaker. If drops of the ether soln are entrapped by the $PbCl_2$ precipitate and are removed with it, decant the soln from the precipitated $PbCl_2$ that has settled into the separatory funnel. Rinse the beaker and precipitate with small quantities of ether, adding the washings to the separatory funnel. Rotate the contents of the separatory funnel and allow to stand for 10 min. Withdraw the aqueous soln and wash the ether with successive 50 cc portions of $H_2O$ until the HCl is removed. Transfer the ether soln to a 300 cc weighed Erlenmeyer flask. Distil the ether and place the flask in an oven heated to about 110° for about 1 hour, while passing a stream of $CO_2$ into the flask to prevent oxidation of the unsaturated acids. Cool in an atmosphere of $CO_2$. When cold, remove the $CO_2$ and weigh. Repeat this treatment until a constant weight is obtained.

Determine in duplicate the I numbers of both the saturated and unsaturated acid fractions. (The I number of the saturated acid fraction is due to the presence of some unsaturated acid.)

To correct for the unsaturated acids present in the fraction of saturated acids use the following formula:

$$\frac{\text{I No. of saturated acid fraction}}{\text{I No. of unsaturated acid fraction}} \times 100 = A \quad \text{(percentage of unsaturated acids in saturated acid fraction).}$$

Obtain the correct value by means of the formula $\frac{A \times B}{100}$, in which $B$ is the percentage of the impure saturated acids (as found by analysis). Subtract this correction from the percentage of impure saturated acids and add it to the percentage of unsaturated acids actually determined. (This method for the separation of the saturated and unsaturated fatty acids is not applicable to the analysis of fats and oils which contain erucic, chaulmoogric, hydnocarpic or similar acids, because the Pb salts of these acids are difficultly soluble in ether. This statement also applies to hydrogenated products which contain notable quantities of iso-oleic acid and to the analysis of coconut or palm kernel oils which contain notable quantities of the lower fatty acids which give ether-soluble Pb salts.)

### 32 FREE FATTY ACIDS—OFFICIAL

Weigh 20 g of fat, or oil, into a flask; add 50 cc of 95% alcohol that has been neutralized with dilute NaOH soln, using phenolphthalein indicator; and heat to boiling. Shake the flask thoroly in order to dissolve the free fatty acids as completely as posible. Titrate with 0.1 $N$ NaOH or KOH, shaking thoroly until the pink color persists after vigorous shaking. Express the results as percentage of oleic acid, as acid degree (cc of 1 $N$ alkali required to neutralize the free acids in 100 g of oil or fat), or as acid value (mg of KOH required to saturate the free acids in 1 g of fat or oil. 1 cc of 0.1 $N$ alkali = 0.0282 g of oleic acid.

## ACETYL VALUE[10]—OFFICIAL

### 33 *Acetylation*

Boil 50 cc of the sample with 50 cc of freshly distilled acetic anhydride under a reflux condenser for 2 hours. Pour the mixture into 500 cc of $H_2O$ in a beaker and boil for 15 min. while bubbling a stream of air or of $CO_2$ thru the soln to prevent bumping. Siphon off the $H_2O$, add 500 cc more of $H_2O$, and boil again for 15 min. Repeat the siphonation and boil for 15 min. with a third 500 cc portion of $H_2O$. Allow the mixture to cool and separate the aqueous layer, which should be neutral to litmus. Transfer the acetylated oil to a separatory funnel and wash with two 200 cc portions of warm $H_2O$. Separate as much of the $H_2O$ as possible, add 5 g of anhydrous $Na_2SO_4$ to the acetylated oil, and let stand for 1 hour, agitating occasionally to assist the drying. Filter thru a dry folded filter, preferably in an oven heated to 100–110°, and keep the filtered oil in the oven until the oil is completely dry. The acetylated product should be a clear, brilliant oil.

### 34 *Saponification*

Weigh accurately 2–2.5 g each of the acetylated oil and of the untreated oil into separate 250 cc Erlenmeyer flasks. Add to each flask exactly 25 cc of alcoholic KOH soln prepared as directed under **21** (**b**), and reflux for 1 hour. Titrate the warm solns with 0.5 $N$ HCl, using phenolphthalein as indicator. Titrate in the same way two 25 cc portions of the alcoholic KOH soln. From the mean of these two results, which should be in very close agreement, deduct the volume of the standard HCl required for the titration of the acetylated and of the untreated oil and from the results so obtained calculate the saponification number (mg of KOH required to saponify 1 g of product) of each. Calculate the acetyl value by means of the following formula:

$$A = \frac{S' - S}{1 - 0.00075S'}, \text{ in which}$$

$A$ = acetyl value;
$S$ = saponification number of oil; and
$S'$ = saponification number of the acetylated oil.

## CHOLESTEROL AND PHYTOSTEROL IN MIXTURES OF ANIMAL AND VEGETABLE FATS

### 35 *Alcohol Extraction Method*[11]*—Tentative*

Introduce 200–300 g of the melted fat into a flat-bottomed liter flask. Close the neck of the flask with a 3-holed stopper and insert thru these holes: (1) A reflux condenser; (2) a right-angled glass tube, one arm of which reaches to a point 6 mm above the surface of the melted fat, the other being closed a short distance from the flask by means of a short piece of rubber tubing and a pinch-cock; (3) a glass tube bent so that one arm reaches down to the bottom of the flask and the other serves as a delivery tube for a 700 cc round-bottomed flask containing 500 cc of 95% alcohol.

Place the flasks containing the melted fat and the alcohol on a steam bath and heat so that the alcohol vapor passes thru the melted fat in the liter flask and is condensed in the reflux condenser, finally collecting in a layer over the melted fat. After all the alcohol has passed in this manner into the flask containing the fat, disconnect the flask from which the alcohol has been distilled and attach a tube to the short piece of rubber tubing attached to the right-angled glass tube, see (2) above, and siphon the alcohol layer back into the alcohol distillation flask. Reconnect as at first and again distil the alcohol as in the first operation. When all the alcohol has been distilled, siphon it again into the distillation flask and extract in the same manner a third time.

Discard the fat and retain the alcohol, which now contains practically all the cholesterol and phytosterol originally present in the fat. Concentrate the alcoholic soln to about 250 cc and to the boiling liquid add 20 cc of KOH soln (1 + 1). Boil for

10 min. to insure complete saponification of the fat, cool to room temp., and pour into a large separatory funnel containing 500 cc of warm ether. Shake to insure thoro mixing and add 500 cc of $H_2O$. Rotate the funnel gently to avoid the formation of extremely stubborn emulsions, but mix the $H_2O$ thoroly with the alcohol-ether-soap soln. A clear, sharp separation takes place at once. Draw off the soap soln and wash the ether layer with 300 cc of $H_2O$, avoiding shaking. Repeat the washing of the ether soln with small quantities of $H_2O$ until all the soap is removed. Transfer the ether layer to a flask and distil the ether until the volume of liquid remaining in the flask measures about 25 cc. Transfer this residue to a tall 50 cc beaker and continue the evaporation until all the ether is driven off and the residue is perfectly dry. If desired, a weighed beaker may be used and the weight of the unsaponifiable matter determined at this point.

Add 3–5 cc of acetic anhydride to the residue in the beaker, cover the beaker with a watch-glass, and heat to boiling over a free flame. After boiling for a few seconds, remove the beaker from the flame, cool, and add 35 cc of alcohol, 60% by volume. Mix the contents of the beaker thoroly, filter off the alcoholic soln and wash the precipitate with the 60% alcohol. Dissolve the precipitate on the filter with a stream of hot alcohol, 80% by volume, and wash the insoluble portion well with the 80% alcohol. Acetates of cholesterol and phytosterol are dissolved, while the greater portion of the impurities present (including paraffin and paraffin oil) remains behind on the filter. Cool the combined filtrate and washings to a temp. of 10–12° and allow to stand at that temp. for 2–3 hours. During this time the acetates of cholesterol and phytosterol crystallize from the soln. Collect the crystals upon a filter, wash with cold alcohol, 80% by volume, and then dissolve in a minimum quantity of hot absolute alcohol. Collect the alcoholic soln of the acetates in a small glass evaporating dish, add 2 or 3 drops of $H_2O$ to the soln, and heat if not perfectly clear. Allow the alcohol to evaporate spontaneously, stirring the contents of the dish occasionally to mix the deposit of crystals that form upon the edges with the main body of the liquid. As soon as a good deposit of crystals has formed, collect them upon a hardened filter; wash twice with cold alcohol, 90% by volume; and dry by suction, drying finally at 100° for 30 min. Determine the melting point in the apparatus shown in the figure under **14,** using $H_2SO_4$ in the outer beaker and glycerin in the inner tube.

The melting point of the first crop of crystals usually gives definite information as to the presence or absence of phytosterol, but the conclusion indicated should be confirmed by recrystallizing the crystals from absolute alcohol and again determining the melting point. If the crystals are pure cholesteryl acetate, the melting point of the second crop should agree closely with that of the first. If phytosteryl acetate is present, however, a higher melting point will be noted, as phytosteryl acetate is less soluble in alcohol than cholesteryl acetate. The melting point of cholesteryl acetate is 114°; that of phytosteryl acetate, 125–137°.

### 36 *Digitonin Method*[12]*—Tentative*

Shake vigorously 50 g of the oil, or fat, for 15 min. in a separatory funnel with 20 cc of a 1% soln of digitonin in 95% alcohol. Allow the mixture to stand for a time until the emulsion separates. The lower or fat layer should be quite clear while the alcohol layer contains a bulky, flocculent precipitate. Draw off as much of the fat as possible, avoiding any loss of the precipitate. Add 100 cc of ether to the alcohol layer and filter the mixture. After drying in the air wash the precipitate with ether until free from fat, transfer to a tall 50 cc beaker, and add 2–3 cc of acetic anhydride. Cover the beaker with a watch-glass. Then boil slowly over a low flame for 30 min. After cooling, add 30–35 cc of alcohol, 60% by volume, and mix the contents of the beaker thoroly. Filter the alcohol soln. Wash the precipitate with the 60% alcohol, then dissolve on the filter with a stream of hot alcohol, 80% by volume, from a wash bottle, and set aside the filtrate in a cool place (10° or below). After the acetates have crystallized out of this soln filter them off, recrystallize from absolute alcohol, dry, and determine the melting point of each crop of crystals as directed under **35**.

### UNSAPONIFIABLE RESIDUE

#### *F. A. C. Method*[13]*—Official*

**37**

#### REAGENT

*Petroleum ether.*—Redistil below 75°. Make a blank determination by evaporating 350 cc of the reagent with about 0.25 g of stearine or other hard fat (previously brought to constant weight by heating) and drying as in the actual determination. The blank must not exceed a few mg.

**38**

#### APPARATUS

*Extraction cylinder.*—Glass-stoppered, graduated at 40 cc, 80 cc, and 130 cc, and of the following dimensions: diameter about 1⅜ inches, height about 12 inches.

**39**

#### DETERMINATION

Weigh 5 g (±0.020 g) of the prepared sample into a 200 cc Erlenmeyer flask, add 30 cc of redistilled 95% (approximately) alcohol (by volume) and 5 cc of 50% aqueous KOH, and boil the mixture for 1 hour under a reflux condenser. Transfer to the extraction cylinder and wash to the 40 cc mark with redistilled 95% alcohol. Complete the transfer, first with warm, then with cold $H_2O$, until the total volume is 80 cc. Rinse the flask with 50 cc of petroleum ether and add the rinsings to the contents of the cylinder previously cooled to room temp. Shake as vigorously as possible for 1 min. and allow to settle until both layers are clear, when the volume of the upper layer should be about 40 cc. Draw off the petroleum ether layer as closely as possible by means of a slender glass siphon into a separatory funnel of 500 cc capacity. Repeat the extraction at least 6 more times, using 50 cc of petroleum ether for each extraction. Wash the combined extracts into a separatory funnel 3 times with 25 cc portions of 10% alcohol by volume, shaking vigorously each time. Transfer the petroleum ether extract to an Erlenmeyer weighed flask and distil; or, if desired, evaporate the petroleum ether on a steam bath in a current of air. Heat the flask with residue until a constant weight is obtained in an oven at a uniform temp. not less than 100° nor more than 110°. (A vacuum oven may be used at a corresponding temp., which depends upon the pressure employed in it. It is important to displace with air any residue vapors of petroleum ether remaining in the flask after heating and before it is weighed.) Deduct any blank from the weight before calculating unsaponifiable matter. Test the final residue for solubility in 50 cc petroleum ether at room temp. Filter, and wash free from the insoluble residue, if any. Evaporate and dry in the same manner as before.

### RESIN OIL

**40**

#### *Qualitative Test—Tentative*

Polarize the pure oil, or a definite dilution, with petroleum ether in a 200 mm tube. Resin oil has a polarization in a 200 mm tube of from +30° to +40° on the sugar scale (Schmidt and Haensch) while most oils[14] read between +1° and −1°.

## COTTONSEED OIL

### 41 *Halphen Test*[15]*—Official*

Mix $CS_2$ containing 1% of S in soln with an equal volume of amyl alcohol. Mix equal volumes of this reagent and the sample under examination and heat in a bath of boiling, saturated brine for 1–2 hours. In the presence of as little as 1% cottonseed oil, a pronounced characteristic red or orange-red color is produced. The depth of color is proportional, to a certain extent, to the quantity of cottonseed oil present, and by making comparative tests with known mixtures of cottonseed oil an approximation of the quantity present can be obtained.

Different oils react with different intensities. Oils that have been heated to 200–210°[16] react with greatly diminished intensity. Heating for 10 min. at 250° renders cottonseed oil incapable of giving the reaction.[17] The fat of animals fed on cottonseed meal or other cottonseed products may give a positive reaction by this test.

## 42 PEANUT OIL[18]—OFFICIAL

Weigh 20 g of the oil into an Erlenmeyer flask. Saponify with alcoholic KOH soln [**21** (b)]; neutralize exactly with acetic acid (1+3), using phenolphthalein indicator; and wash into an 800–1000 cc flask containing a boiling mixture of 100 cc of $H_2O$ and 120 cc of 20% Pb-acetate soln. Boil for a min. and then cool the precipitated soap by immersing the flask in $H_2O$, occasionally giving it a whirling motion to cause the soap to stick to the sides of the flask. After the flask has cooled, decant the $H_2O$ and excess of Pb-acetate soln and wash the Pb soap with cold $H_2O$ and alcohol, 90% by volume. Add 200 cc of ether, cork, and allow to stand until the soap is disintegrated; heat on a water bath, using a reflux condenser, and boil for about 5 min.[18] In the case of oils, most of the soap will be dissolved, while in lards, which contain much stearin, part of the soap will be left undissolved. Cool the ether soln of soap to 15–17° and allow to stand until all the insoluble soaps have separated out (about 12 hours).

Filter upon a Büchner funnel and thoroly wash the insoluble Pb soaps with ether. Wash the ether-insoluble Pb soaps into a separatory funnel by means of a jet of ether, alternating at the end of the operation if a little of the soap sticks to the paper with HCl (1+3). Add sufficient HCl (1+3) so that the total volume of the acid amounts to about 200 cc and enough ether to make its total volume 150–200 cc and shake vigorously for several min. Allow the layers to separate, run off the acid layer, and wash the ether once with 100 cc of the dilute HCl and then with several portions of $H_2O$ until the $H_2O$ washings are no longer acid to methyl orange. If a few undecomposed lumps of Pb soap remain (indicated by solid particles remaining after the third washing with $H_2O$), break these up by running off almost all the water layer and then add a little HCl; shake; and continue the washing with $H_2O$ as before. Distil the ether from the soln of insoluble fatty acids and dry the latter in the flask by adding a little absolute alcohol and evaporating on a steam bath. Dissolve the dry fatty acids by warming with 100 cc of 90% alcohol by volume. Cool slowly to 15°, shaking to aid crystallization. Allow to stand at 15° for 30 min. In the presence of peanut oil, crystals of arachidic acid will separate from the soln. Filter, and wash the precipitate twice with 10 cc of alcohol, 90% by volume, and then with alcohol, 70% by volume, taking care to maintain the arachidic acid and the wash solns at a definite temp. in order to apply the solubility corrections given below. Dissolve the arachidic acid upon the filter with boiling absolute alcohol, evaporate to dryness in a weighed dish, dry, and weigh. Add to the weight 0.0025 g for each 10 cc of 90% alcohol used in the crystallization and washing, if conducted at 15°; if conducted at 20°, add 0.0045 g for each 10 cc. The melting point of arachidic acid thus obtained is 71–72. Twenty times the weight of arachidic acid will give the approximate quantity of peanut oil present. Arachidic acid has a characteristic appearance and may be identified under the microscope. As little as 5–10% of peanut oil can be detected by this method.

### 43 COLD TEST[20]—OFFICIAL, FIRST ACTION

(Applicable to all salad oils except olive oil)

Fill a 4-ounce sample bottle with the oil at a temp. of 25°, insert the cork stopper tightly, and seal with paraffin. Submerge the bottle completely in a bucket containing finely cracked ice and add $H_2O$ until it rises to the top of the bottle. Keep the bucket filled solidly with the cracked ice by removing any excess $H_2O$ and adding ice when necessary. At the end of 5 hours remove the bottle and examine the oil. If it is properly wintered, the sample will be brilliant, clear, and limpid.

## SESAME OIL

### 44 *Baudouin Test—Official*

Dissolve 0.1 g of finely powdered sugar in 10 cc of HCl, add 10 cc of the oil to be tested, shake thoroly for 1 min., and allow to stand for 10 min. In the presence of even a very small admixture of sesame oil, the aqueous soln is colored crimson. It should be observed that some olive oils, especially those of African or Spanish origin, give pink or crimson colors. These can be readily differentiated from the color due to sesame oil by the modified Villavecchia test, **45.**

### 45 *Modified Villavecchia Test[21]—Official*

Add 2 cc of furfural to 100 cc of 95% alcohol. Mix thoroly 0.1 cc of this soln with 10 cc of HCl and 10 cc of the oil to be tested by shaking them together in a test tube for 15 seconds. Allow the mixture to stand for 10 min., observe color, add 10 cc of $H_2O$, shake, and again observe the color. If the crimson color disappears, sesame oil is not present. (As furfural gives a violet tint with HCl, it is necessary to use the very dilute soln specified.)

## DETECTION OF FOREIGN FATS CONTAINING TRISTEARIN IN LARD

### 46 *Method I[22]—Tentative*

Weigh 5 g of the melted fat into a glass-stoppered 25 cc cylinder about 150–175 mm in height. Add warm ether to the 25 cc mark, stopper securely, and shake until the fat is completely dissolved. Allow the cylinder to stand for about 18 hours at a temp. of 16–20°, during which time some of the solid glycerides will crystallize out. Decant the clear soln carefully from the crystals and wash with three 5 cc portions of cold ether, taking care to avoid breaking up the deposit during the first two washings. Agitate the crystals with the third portion of ether and transfer to a small filter. Wash on the paper with successive small quantities of cold ether until 15–20 cc has been used; then remove the last traces of ether by means of slight suction on the stem of the funnel. Break up any large lumps and allow the deposit to dry thoroly. Pulverize the glycerides and take their melting point in a closed 1 mm tube, using an apparatus similar to that indicated under **14.** Heat the $H_2O$ in the beaker rapidly to about 55° and maintain this temp. until the thermometer carrying the melting-point tube registers 50–50°; then heat again and carry the temp. of the outer bath somewhat rapidly to 67°. Remove the burner. The melting point is reached when the fused substance becomes perfectly clear and transparent. A dark background placed about 4 inches from the apparatus will be helpful. When the melting point of the glycerides obtained by this method is below 63.4°, the presence of beef fat should be suspected; a melting point of 63°, or below, is positive evidence that the sample is not pure lard. It is advisable to carry out this method with a control sample of pure lard.

### 47 *Method II—Tentative*

Weigh 5 g of the filtered fat into a glass-stoppered cylinder graduated to 25 cc and add warm acetone until the 25 cc mark is reached. Shake the cylinder until the contents are thoroly mixed and allow to stand where a temp. of 30° is maintained. After 18 hours remove the cylinder and carefully decant the supernatant acetone soln from the crystallized glycerides, which are usually found in a firm mass at the bottom of the cylinder. Add warm acetone in 3 portions of 5 cc each from a small wash bottle, taking care not to break up the deposit while washing, and decant the first 2 portions. Actively agitate the third portion in the cylinder, and by a quick movement transfer with the crystals to a small filter paper. Using the wash bottle, wash the crystals with 5 successive small portions of the warm acetone, and remove the excess acetone by suction. Transfer the paper with its contents to a suitable place, spread out, and break up by gentle pressure any large lumps of the glycerides. When dry, thoroly comminute the mass and determine the melting point of the crystals in a closed 1 mm tube as directed under **46.** A melting point below 63° is regarded as evidence of adulteration, and a melting point below 63.4° is regarded as suspicious.

After determining the melting point, transfer the crystallized glycerides to a 50 cc beaker, add 25 cc of approximately 0.5 *N* alcoholic KOH, and heat on a steam bath until saponification is complete. Pour the soln into a separatory funnel containing 200 cc of $H_2O$, acidify, add 75 cc of ether, and shake. Draw off the acid layer and wash at least 3 times with $H_2O$. Transfer the ether soln to a clean dry 50 cc beaker, drive off the ether on a steam bath, and finally dry the acids at 100°. After about 2 hours determine the melting point as directed under **46.** If the melting point of the glycerides, plus twice the difference between the melting point of the glycerides and the melting point of the fatty acids, is less than 73°, the lard is regarded as adulterated.

## FISH OIL AND MARINE ANIMAL OILS IN THE PRESENCE OF VEGETABLE OILS AND IN THE ABSENCE OF METALLIC SALTS

### 48 *Qualitative Test—Tentative*

Dissolve in a test tube about 6 g of the oil in 12 cc of a mixture of equal parts of $CHCl_3$ and glacial acetic acid. Add Br, dropwise, until a slight excess is indicated by the color, keeping the soln at about 20°. Allow the mixture to stand 15 min. or more and then place the test tube in boiling $H_2O$. If vegetable oils only are present, the soln will be perfectly clear, but fish oils will remain cloudy owing to the presence of insoluble bromides.

## 49 COLORING MATTERS—TENTATIVE

Proceed as directed under **XXI.**

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# XXXII. PRESERVATIVES AND ARTIFICIAL SWEETENERS

## SALICYLIC ACID

### 1 PREPARATION OF SAMPLE—OFFICIAL

(a) *Non-alcoholic liquids.*—Many liquids may be extracted directly as described under **2** or **4** without further treatment. If troublesome emulsions form during extraction, pipet 100 cc into a 250 cc volumetric flask, and add about 5 g of NaCl, shaking until dissolved. Make up to the mark with 95% alcohol, shake vigorously, allow the mixture to stand for 10 min. with occasional shaking, filter, and treat an aliquot of the filtrate as directed under (**b**).

(**b**) *Alcoholic liquids.*—Make 200 cc of the sample alkaline with approximately 10% NaOH soln, using litmus paper as an indicator, and evaporate on a steam bath to about ⅓ its original volume. Dilute to the original volume with $H_2O$ and filter, if necessary.

(**c**) *Solid or semi-solid substances.*—Grind the sample and mix thoroly. Transfer a convenient quantity (50–200 g according to the consistency of the sample) to a 500 cc volumetric flask; add sufficient $H_2O$ to make a volume of about 400 cc; and shake until the mixture becomes uniform. Add 2–5 g of $CaCl_2$ and shake until dissolved; render distinctly alkaline with approximately 10% NaOH soln, using litmus paper as an indicator; fill to the mark with $H_2O$; shake thoroly; allow to stand for at least 2 hours, shaking frequently; and filter.

### QUALITATIVE TESTS

### 2 *Ferric Chloride Test—Official*

Introduce 50 cc of the sample or an equivalent quantity of an aqueous extract, prepared as directed under **1**, into a separatory funnel; add 1/10 its volume of HCl (1+3); and extract with 50 cc of ether. If the mixture emulsifies, add 10–15 cc of petroleum ether (b.p. below 60°) and shake. If this treatment fails to break the emulsion, whirl the mixture in a centrifuge, or allow it to stand until a considerable portion of the aqueous layer has separated; run off the latter; shake vigorously; and again allow to separate. Wash the ether layer with two 5 cc portions of $H_2O$, evaporate the greater portion of the ether in a porcelain dish on a steam bath, allow the remainder to evaporate spontaneously, and add a drop of 0.5% neutral $FeCl_3$ soln. A violet color indicates salicylic acid.

If coloring matter or other interfering substance is present in the residue left after evaporation of the ether, purify the salicylic acid by one of the following methods:

(**a**) Dissolve the original residue from the ether extract, obtained as directed above, in about 25 cc of ether; transfer this soln to a separatory funnel; and shake with an equal volume of $H_2O$ made distinctly alkaline with several drops of $NH_4OH$. Allow to separate, filter the aqueous layer thru a wet filter into a porcelain dish, evaporate almost to dryness, and test the residue as directed previously.

(**b**) Dry the original residue from the ether extract, obtained as directed above, in a desiccator over $H_2SO_4$ and extract with several 10 cc portions of $CS_2$ or petroleum ether (b. p. below 60°), rubbing the contents of the dish with a glass rod and filtering the successive portions of the solvent thru a dry paper into a second porcelain dish. Evaporate the greater portion of the solvent on a steam bath, allow the remainder to evaporate spontaneously, and test the residue as directed previously.

**(c)** By means of a few cc of ether, transfer the original residue from the ether extract, obtained as directed above, to a small porcelain crucible, and allow the solvent to evaporate spontaneously. Cut a hole sufficiently large in an asbestos board to admit about $\frac{2}{3}$ of the crucible, cover with a small, round-bottomed flask filled with cold $H_2O$, and heat over a small Bunsen flame until any salicylic acid present has sublimed and condensed upon the bottom of the flask. Test the sublimate as directed previously.

### 3 *Jorissen Test*[1]*—Official*

Dissolve the residue from the ether extract, obtained as directed under **2**, or, if impurities are present, the purified material obtained as directed under **2** (**a**), (**b**), or (**c**), in a little hot $H_2O$. Cool 10 cc of the soln in a test tube; add 4 or 5 drops of 10% $KNO_2$ soln, 4 or 5 drops of acetic acid (approximately a 50% soln) and 1 drop of one per cent* $CuSO_4$ soln; mix thoroly; boil the liquid for half a min.; and allow to stand for 2 min. In the presence of salicylic acid a Bordeaux-red color develops.

## QUANTITATIVE METHOD—OFFICIAL

### 4 EXTRACTION

Transfer 100 cc of the sample, or that quantity of a soln prepared as directed under **1** which represents not less than 20 g of the original material, to a separatory funnel; neutralize to litmus with HCl (1+3); and add an excess of HCl equivalent to 2 cc of acid for each 100 cc of soln. Extract with 4 separate portions of ether, using for each extraction a volume of ether equivalent to $\frac{1}{2}$ the volume of the aqueous layer. If an emulsion forms on shaking, this may usually be broken by adding a little ($\frac{1}{5}$ the volume of the ether layer) petroleum ether (b. p. below 60°) and shaking again, or by centrifugalizing. If a small quantity of emulsion still persists, allow it to remain with the aqueous layer, where it is frequently broken during the next extraction. If an emulsion remains after the fourth extraction, separate it from the clear ether and the clear aqueous layer and extract it separately with 2 or 3 small portions of ether. Combine the ether extracts, wash with a volume of $H_2O$ equal to 1/10 of the total volume of ether extracts, allow the layers to separate, and reject the aqueous layer. Wash in this way until the aqueous layer after separation yields a yellow color upon the addition of methyl orange and 2 drops of 0.1 *N* NaOH. Distil slowly the greater part of the ether, transfer the remainder to a porcelain dish, and allow it to evaporate spontaneously. If no interfering substances are present, proceed as directed under **5**; if interfering substances are present, purify the residue by one of the following methods:

**(a)** Thoroly dry the residue in vacuo over $H_2SO_4$. Extract it 10 times with 10–15 cc portions each of $CS_2$ or petroleum ether (b. p. below 60°), rubbing the contents of the dish with a glass rod, and filter the successive portions of the solvent thru a dry filter into a porcelain dish. Test the extracted residue with a drop of 2% ferric alum soln, and if it gives a reaction for salicylic acid, dissolve it in $H_2O$; acidify the soln with HCl (1+3); extract with ether; evaporate; extract the dry residue thus obtained with $CS_2$ or petroleum ether; and add to the extract first obtained. Distil the greater portion of the $CS_2$ or petroleum ether and allow the remainder to evaporate spontaneously. Proceed as directed under **5**.

**(b)** Dissolve the residue in 40–50 cc of ether. Transfer the ether soln to a separatory funnel and extract with 3 successive 15 cc portions of 1% $NH_4OH$. (If fat is known to be present in the original ether extract, extract the latter directly with 4 portions of the $NH_4OH$ instead of 3.) Combine the alkaline aqueous extracts, acidify, again extract with ether, and wash the combined ether extracts as directed previously. Distil slowly the greater portion of the ether, allow the remainder to evaporate spontaneously, and proceed as directed under **5**.

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* The change from "10%" to "1%" has been adopted as official, first action.

### 5 DETERMINATION

Dissolve the residue, obtained under **4**, in a small quantity of hot $H_2O$, and after cooling dilute to a definite volume (usually 50 or 100 cc). If the soln is not clear, filter thru a dry filter. Dilute aliquots of the soln and treat with 0.5% $FeCl_3$ soln or 2% ferric alum soln until the maximum color is developed. Generally a few drops will suffice.

(The ferric alum soln should be boiled until a precipitate appears, allowed to settle, and filtered. The acidity of the soln is slightly increased in this manner, but the soln remains clear for a considerable time, and the turbidity caused by its dilution with $H_2O$ is much less and does not appear so soon as when the unboiled soln is used. This turbidity interferes with the exact matching of the color.)

Compare the colors developed with the color obtained when a standard salicylic acid soln (containing 1 mg of salicylic acid in 50 cc) is similarly treated, using Nessler tubes or a colorimeter. In either case, and especially with $FeCl_3$, avoid an excess of the reagent, altho an excess of 0.5 cc of 2% ferric alum soln may be added to 50 cc of the comparison soln of salicylic acid without vitiating the results.

## BENZOIC ACID

### QUALITATIVE TESTS

### 6 *Preliminary Test—Official*

Extract benzoic acid as directed for salicylic acid under **2** or **4**. If benzoic acid is present in considerable quantity, it will crystallize from the ether in shining leaflets having a characteristic odor on warming. Dissolve the crystalline deposit in hot $H_2O$, divide into 2 portions, and test according to **7** or **8**. The deposit may also be purified as directed under **2** (**c**) and the melting point determined.

### 7 *Ferric Chloride Test—Official*

Make the soln from **6** alkaline with a few drops of $NH_4OH$, expel the excess of $NH_3$ by evaporation, dissolve the residue in a few cc of hot $H_2O$, filter if necessary, and add a few drops of a neutral 0.5% $FeCl_3$ soln. A brownish precipitate of ferric benzoate indicates the presence of benzoic acid.

### 8 *Modified Mohler Test[2]—Official*

(The presence of phenolphthalein interferes with this test)

Add to the water soln, prepared as described under **6**, 1 or 2 drops of about 10% NaOH soln and evaporate to dryness. To the residue add 5–10 drops of $H_2SO_4$ and a small crystal of $KNO_3$. Heat for 10 min. in a bath (glycerol) at 120–130° (the temp. must not exceed 130°). After cooling, add 1 cc of $H_2O$ and make distinctly ammoniacal. Boil the soln to decompose any $NH_4NO_2$ that might have been formed. Cool, and add a drop of fresh, colorless $(NH_4)_2S$ soln, but do not allow the layers to mix. A red-brown ring indicates benzoic acid. On mixing the color diffuses thru the whole liquid, and on heating finally changes to greenish yellow. This change differentiates benzoic acid from salicylic acid or cinnamic acid. The salicylic and cinnamic acids form colored compounds that are not destroyed by heating.

## QUANTITATIVE METHODS—OFFICIAL

### PREPARATION OF SAMPLE

### 9 *General Method*

Mix the sample thoroly, grinding if solid or semi-solid. Transfer 150 cc or 150 g to a 500 cc volumetric flask, add enough pulverized NaCl to saturate the $H_2O$ in the sample, make alkaline to litmus paper with 10% NaOH soln or with milk of lime, and dilute to the mark with saturated NaCl soln. Shake thoroly, allow to stand for at least 2 hours, with frequent shaking, and filter.

If the sample contains large quantities of fat, portions of which may contaminate the filtrate, add a few cc of the NaOH soln to the filtrate and extract with ether before proceeding as directed under **11.** If alcohol is present, proceed as directed under **10** (**c**). If the sample contains large quantities of matter precipitable by salt soln, proceed as directed under **10** (**d**).

### 10 *Special Methods*

(**a**) *Ketchup.*—To 150 g of the ketchup add 15 g of pulverized NaCl, and transfer the mixture to a 500 cc volumetric flask, rinsing with about 150 cc of saturated NaCl soln. Make slightly alkaline to litmus paper with 10% NaOH soln and fill to the mark with saturated NaCl soln. Allow to stand for at least 2 hours, shaking frequently. Squeeze thru a heavy muslin bag and filter.

(**b**) *Jellies, jams, preserves, and marmalades.*—Digest 150 g of the sample in about 300 cc of saturated NaCl soln. Add 15 g of pulverized NaCl. Make alkaline to litmus paper with milk of lime. Transfer to a 500 cc volumetric flask and dilute to the mark with saturated salt soln. Allow to stand for at least 2 hours, shaking frequently; centrifugalize if necessary; and filter.

(**c**) *Cider containing alcohol, and similar products.*—Make 250 cc of the sample alkaline to litmus paper with 10% NaOH soln and evaporate on a steam bath to about 100 cc. Transfer the sample to a 250 cc volumetric flask, add 30 g of pulverized NaCl, and shake until dissolved. Dilute to the original volume, 250 cc, with saturated salt soln; allow to stand for at least 2 hours, shaking frequently; and filter.

(**d**) *Salted or dried fish.*—Wash 50 g of the ground sample into a 500 cc volumetric flask with $H_2O$. Make slightly alkaline to litmus paper with 10% NaOH soln and dilute to the mark with $H_2O$. Allow to stand for at least 2 hours, shaking frequently, and filter. Pipet as large a measured portion of the filtrate as possible (at least 300 cc) into a second 500 cc flask, adding 30 g of the pulverized NaCl for each 100 cc of soln. Shake until the salt has dissolved and dilute to the mark with saturated salt soln. Mix thoroly and filter off the precipitated protein and other extraneous matter.

### 11 DETERMINATION

Pipet a convenient portion (100–200 cc) of the filtrate obtained under **9** or **10** into a separatory funnel. Neutralize the soln to litmus paper with HCl (1+3) and add an excess of 5 cc of the same acid. In the case of salted fish a precipitation of protein matter usually occurs on acidifying, but the precipitate does not interfere with the extraction. Extract carefully with $CHCl_3$, using successively portions of 70, 50, 40, and 30 cc. To avoid the formation of an emulsion, shake cautiously each time using a rotary motion. The $CHCl_3$ layer usually separates readily after standing a few min. If an emulsion forms, break it by stirring the $CHCl_3$ layer with a glass rod, by drawing it off into a second funnel and giving one or two sharp shakes from one end of the funnel to the other, or by centrifugalizing for a few min. As this is a progressive extraction, draw off carefully as much of the clear $CHCl_3$ soln as possible after each extraction, but do not draw off any of the emulsion with the $CHCl_3$ layer. If this precaution is taken, the $CHCl_3$ extract need not be washed.

Transfer the combined $CHCl_3$ extracts to a porcelain evaporating dish, rinse the container several times with a few cc of $CHCl_3$, and evaporate to dryness at room temp. in a current of dry air.

The extract may also be transferred from the separatory funnel to a 300 cc Erlenmeyer flask, the separatory funnel being rinsed 3 times with 5–10 cc portions of $CHCl_3$. Distil very slowly at a low temp. to about $\frac{1}{4}$ the original volume. Then transfer the residue to a porcelain evaporating dish, rinsing the flask 3 times with 5–10 cc portions of $CHCl_3$, and evaporate to dryness at room temp. in a current of dry air.

Dry the residue overnight (or until no odor of acetic acid can be detected if the product is a ketchup) in a desiccator containing $H_2SO_4$. Dissolve the residue of benzoic acid in 30–50 cc of 95% alcohol neutral to phenolphthalein; add about $\frac{1}{4}$ this volume of $H_2O$ and 1 or 2 drops of phenolphthalein indicator [**II, 8 (d)**]; and titrate with 0.05 *N* NaOH. 1 cc of 0.05 *N* NaOH = 0.0072 g of anhydrous Na-benzoate.

## SACCHARIN

### 12 PREPARATION OF SAMPLE

(**a**) *Fruit juices, sirups, and other non-alcoholic liquids.*—Transfer 100–200 g of the sample to a 250 cc volumetric flask by means of a little $H_2O$, dilute to about 200 cc with $H_2O$, add 5 cc of glacial acetic acid, mix, add a slight excess of 20% neutral Pb-acetate soln, mix thoroly, dilute to the mark with $H_2O$, again mix thoroly, and filter.

(**b**) *Alcoholic liquids.*—Heat 100–200 cc of the liquid on a steam bath to remove alcohol, this being accomplished in most cases by evaporating to $\frac{1}{2}$ the original volume. With heavy sirups, dilute the liquid with an equal volume of $H_2O$ before beginning the evaporation. After the alcohol has been removed, transfer to a 250 cc volumetric flask and proceed from this point as directed under (**a**).

(**c**) *Solid or semi-solid preparations.*—Transfer 50–75 g of the sample to a 250 cc volumetric flask by means of a little hot $H_2O$ and add sufficient boiling $H_2O$ to make the volume about 200 cc. Allow the mixture to stand for 2 hours, shaking occasionally. Then add 5 cc of glacial acetic acid, mix thoroly, add a slight excess of 20% neutral Pb-acetate soln, dilute to the mark with cold $H_2O$, mix, allow to stand for 20 min., and filter.

### 13 QUALITATIVE TEST—OFFICIAL

(**a**) Acidify 50 cc of non-alcoholic liquid foods or the aqueous extract of 50 g of a solid or semi-solid product, prepared as directed under **12**, with HCl and extract 3 times with 25 cc portions of ether. Wash the combined ether extracts once with 5 cc of $H_2O$, transfer to a small beaker or evaporating dish, allow the ether to evaporate spontaneously, and taste the residue. The presence of as little as 20 mg of saccharin per liter or kg of the original sample can usually be detected by its sweet taste. Confirm by heating with NaOH and detecting the salicylic acid formed thereby as directed under (**b**).

(**b**) Acidify 50 cc of a non-alcoholic liquid food, or an equivalent quantity of an aqueous extract prepared as directed under **12**, with HCl and extract with 3 portions of ether as directed under (**a**). Dissolve the residue remaining after evaporation of the ether in a little hot $H_2O$ and test a small portion of the soln for salicylic acid as directed under **2** or **3**. Dilute the remainder of the soln to about 10 cc and add 2 cc of $H_2SO_4$ (1+3). Heat to boiling and add a slight excess of 5% $KMnO_4$ soln dropwise; partly cool the soln; dissolve approximately 1 g of NaOH in it; and filter the mixture into an Ag dish (silver crucible lids are well adapted to the purpose); evaporate to dryness; and heat for 20 min. at 210–215°. Dissolve the residue in $H_2O$, acidify with HCl, and test the ether extract for salicylic acid as directed under **2** or **3**. By this method all the so-called "false saccharin" and any salicylic acid naturally present (also added salicylic acid when not present in too large a quantity) are destroyed, whereas 5 mg of saccharin per liter is detected with certainty.

### 14 QUANTITATIVE METHOD—OFFICIAL

Transfer 150 cc of the filtrate obtained under **12** to a separatory funnel, add 15 cc of HCl, and extract 3 times with 80 cc portions of ether, shaking the separatory funnel for 2 min. each time. Wash the combined ether extracts once with 5 cc of $H_2O$, remove the ether by distillation, and transfer the residue to a Pt crucible by means of a little ether; or, if substances difficultly soluble in ether are present, use alternately small portions of $H_2O$ and ether. Evaporate the ether on a steam bath, add to the residue 2–3 cc (or enough to make the mixture strongly alkaline) of a 10% $Na_2CO_3$ soln, rotate so that all the saccharin is brought in contact with the soln, and evaporate to dryness on a steam bath. To the dry residue in the crucible add 4 g of a mixture of equal parts of anhydrous $Na_2CO_3$ and $K_2CO_3$. Heat gently at first and then to complete fusion for 30 min. over an alcohol or other S-free flame. The fusion may be conducted by closely fitting the crucible into a hole cut into a piece of heavy asbestos board so that $\frac{1}{3}$ of the crucible projects above the asbestos, and heating the lower portion of the crucible by means of a large Bunsen, Meker, or similar burner. Cool, dissolve the melt in $H_2O$, add about 5 cc of Br water, acidify with HCl, filter, wash the paper with a little $H_2O$, dilute the filtrate and washings to about 200 cc, heat to boiling, and slowly add an excess of $BaCl_2$ soln (approximately 10%). Allow to stand overnight, collect the $BaSO_4$ on a filter or on a Pt Gooch crucible, wash until free from chlorides, dry, ignite, cool, and weigh. Correct the result thus obtained for any S present in the fusion mixture as found by a blank determination. Calculate the equivalent quantity of saccharin by multiplying the corrected weight of $BaSO_4$ by 0.7844.

Instead of the mixed Na- and K-carbonates, 3–4 g of $Na_2O_2$ may be employed for the fusion. In this case a Ni crucible must be used, and the time of fusion may be reduced to 5 min. The separation of a little $PbCl_2$ during the extractions does not interfere with the accuracy of the method.

## BORIC ACID AND BORATES

### 15 QUALITATIVE TEST[3]—OFFICIAL

*Preliminary test.*—Acidify the sample with HCl in the proportion of 7 cc of acid to each 100 cc of sample. In case of solid or pasty samples heat with enough $H_2O$ to make sufficiently fluid before acidifying. Immerse a strip of turmeric paper in the acidified liquid, and allow the paper to dry spontaneously. If borax or $H_3BO_3$ is present, the paper will acquire a characteristic red color, changed by $NH_4OH$ to a dark blue-green, but restored by acid.

*Confirmatory test.*—Make about 25 g of the sample decidedly alkaline with lime $H_2O$ and evaporate to dryness on a steam bath. Ignite the dry residue at a low red heat until the organic matter is thoroly charred, cool, digest with about 15 cc of $H_2O$, and add HCl, dropwise, until the soln is distinctly acid. Immerse a piece of turmeric paper in the soln and allow it to dry without heat. In the presence of borax or $H_3BO_3$, the color change will be the same as described under the preliminary test.

### 16 QUANTITATIVE METHOD[4]—OFFICIAL

Make 10–100 g of the sample (depending upon the nature of the material and the quantity of $H_3BO_3$ present) distinctly alkaline with NaOH soln and evaporate to dryness in a Pt dish. Ignite the residue until the organic matter is thoroly charred, avoiding an intense red heat, cool, digest with about 20 cc of hot $H_2O$, and add HC dropwise until the reaction is distinctly acid. Filter into a 100 cc volumetric flask and wash with a little hot $H_2O$. The volume of the filtrate should not exceed 50–60 cc. Return the filter containing any unoxidized C to the Pt dish, make alkaline by wetting thoroly with lime $H_2O$, dry on a steam bath, and ignite to a white ash. Dissolve the ash in a few cc of HCl (1+3) and add to the liquid in the 100 cc flask, rinsing the dish with a few cc of $H_2O$. To the combined solns, add 0.5 g of $CaCl_2$ and a few drops of phenolphthalein indicator, then 10% NaOH soln until a permanent light pink color is produced, and finally dilute to the mark with lime $H_2O$. Mix, and filter thru a dry filter. To 50 cc of the filtrate add 1 *N* $H_2SO_4$ [**II, 17** (**b**)], until the pink color disappears, then add methyl orange indicator [**VI, 3** (**f**)], and continue the addition of the acid until the yellow color is changed to pink. Boil for about 1 min. to expel $CO_2$. Cool, and carefully add 0.2 *N* NaOH until the liquid assumes a yellow tinge, avoiding an excess of the alkali. All the boric acid is now in a free state with no uncombined $H_2SO_4$ present. Add about 10 g of neutral mannitol and a few drops of phenolphthalein indicator, note the buret reading, and again titrate the soln with the standard NaOH until a pink color develops. Add a little more mannitol, and if the pink color disappears continue the addition of the standard alkali until a pink color reappears. Repeat the alternate addition of mannitol and standard alkali until a permanent end point is reached. A volume of neutral glycerol equal to the volume of the soln to be titrated may be substituted for the mannitol. 1 cc of 0.2 *N* NaOH soln = 0.0124 g of boric acid.

## FORMALDEHYDE

### 17 PREPARATION OF SAMPLE—OFFICIAL

If the sample is solid or semi-solid, macerate 200–300 g of the material with about 100 cc of $H_2O$ in a mortar. Transfer to a short-necked, 500–800 cc Cu or glass distillation flask, make distinctly acid with $H_3PO_4$, connect with a condenser, and distil 40–50 cc. With highly colored liquids, make about 200 cc distinctly acid with $H_3PO_4$ and distil as directed previously.

### QUALITATIVE TESTS

### 18 *I. Phenylhydrazin Hydrochloride Test[5]—Official*

With milk and other liquids, shake with an equal volume of 95% alcohol, filter, and use the filtrate. With meats and fats, extract the HCHO with alcohol and use the filtrate. With fat, heat the mixture above the melting point of the fat to insure thoro extraction.

Mix 5 cc of the distillate obtained under **17**, or of an alcoholic soln or extract, obtained as directed previously, with 0.03 g of phenylhydrazin hydrochloride and 4 or 5 drops of a 1% $FeCl_3$ soln. Add slowly and with agitation, in a bath of cold $H_2O$ to prevent heating the liquid, 1–2 cc of $H_2SO_4$. Dissolve the precipitate by the addition of either $H_2SO_4$ (keeping the mixture cool) or alcohol. In the presence of HCHO a red color develops.

This method gives reliable reactions for HCHO in solns of HCHO varying from 1 part in 50,000 to 1 part in 150,000. Neither acetaldehyde nor benzaldehyde interferes with the reaction.

### 19 *II. Hehner Test[6]—Official*

Mix about 5 cc of the distillate obtained under **17** with an equal volume of pure milk or with a 1–2% soln of egg albumin, in a test tube and underlay with commercial $H_2SO_4$ without mixing. A violet or blue color at the junction of the two liquids indicates HCHO. This color is given only in the presence of a trace of $FeCl_3$ or other oxidizing agent. If only pure acid is available, add a few drops of $FeCl_3$ soln to the acid before making the test. Milk may be treated directly by this method, and it gives positive tests in the presence of one or more parts of HCHO per 10,000. Other articles of food rich in proteins, for example, egg albumin, give the reaction in the presence of $H_2O$ without the addition of milk.

### 20 *III. Leach Test—Official*

Mix in a porcelain casserole about 5 cc of the distillate obtained under **17** with an equal volume of pure milk and add about 10 cc of HCl soln containing 2 cc of 10% $FeCl_3$ soln to each liter of acid. Heat to 80–90° directly over the gas flame, rotating the casserole to break up the curd. A violet coloration indicates HCHO.

### 21 *IV. Phenylhydrazin Hydrochloride and Sodium Nitroprusside Test[7]—Official*

This test may be applied directly to liquid foods, to an aqueous or alcoholic extract of solid foods, or to the distillate prepared as directed under **17.** In the case of milk, apply the method directly. With meat, comminute the sample, extract with 2 volumes of hot $H_2O$, and use the expressed liquid for the test. Heat approximately 10 g of fats above their melting point with 20 cc of alcohol, shake thoroly, cool, filter thru a moistened filter, and use the filtrate for the test.

Dissolve a lump of phenylhydrazin hydrochloride about the size of a pea in 3–5 cc of the liquid to be tested, and add 2–4 drops (not more) of a 5–10% Na-nitroprusside soln and 8–12 drops of an approximately 10% NaOH soln. If HCHO is present, a green or blue color, depending upon the quantity, develops. When HCHO is present to the extent of more than 1 part in 70,000–80,000 in the soln tested, a distinct green or bluish green coloration is obtained. In more dilute solns the green tint becomes less marked, and a yellow tinge tending toward greenish brown develops. With this test acetaldehyde and benzaldehyde give a color varying, according to the strength of the soln, from red to brown. Therefore, a reaction may be obtained with these aldehydes similar to that obtained with HCHO in solns more dilute than 1 part in 70,000. The presence of acetaldehyde or benzaldehyde together with HCHO gives a yellowish or yellowish green tinge. The reaction for HCHO, therefore, may be masked by the presence of other aldehydes, but it is characteristic when a clear green color is obtained.)

### 22 *V. Phenylhydrazin Hydrochloride and Potassium Ferricyanide Test[7]—Official*

Proceed as directed under **21**, substituting a soln of $K_3Fe(CN)_6$ for the Na-nitroprusside. HCHO gives a red color. Alcoholic extracts from foods must be diluted with $H_2O$ to prevent the precipitation of $K_3Fe(CN)_6$. The test is not applicable in the presence of the coloring matter of blood.

### 23 *VI. Phenylhydrazin Hydrochloride and Ferric Chloride Test[7]—Official*

Treat 15 cc of milk or other liquid food or of the distillate prepared as directed under **17** with 1 cc of 1% phenylhydrazin hydrochloride soln, then with a few drops of 1% $FeCl_3$ soln, and finally with HCl. The presence of HCHO is indicated by the formation of a red color, which changes after some time to orange yellow. Milk may be examined directly by this method, but more delicate tests may be obtained from the distillate from milk or from milk serum. Acetaldehyde or benzaldehyde does not interfere with the reaction.

### 24 *VII. Phloroglucol Test[8]—Official*

To 10 cc of milk or other liquid food under examination in a test tube add, by means of a pipet, 2 cc of phloroglucol reagent (1 g of phloroglucol, 20 g of NaOH, and $H_2O$ to make 100 cc), placing the end of the pipet on the bottom of the tube in such a manner that the reagent will form a separate layer. If HCHO is present, a bright red coloration (not purple) forms at the zone of contact.

(This soln gives a yellow color in the presence of some aldehydes, and if it is used for the detection of aldehyde formed by the oxidation of methyl alcohol after the destruction of acetaldehyde with $H_2O_2$ soln, an orange yellow color will slowly appear when an insufficient quantity of $H_2O$ soln has been employed. On the other hand, if the excess of $H_2O_2$ soln is not fully destroyed before the use of this reagent, a purple color develops slowly. The clear, red color given by the use of this reagent forms quickly, and in the presence of but a small quantity of HCHO it fades rapidly.)

## SOLUBLE FLUORIDES

### 25 QUALITATIVE TEST I[9]—OFFICIAL

(Not applicable in the presence of silica)

After thoroly mixing the sample transfer 150 cc, or an equivalent quantity of an aqueous extract in the case of solid foods, to a beaker and boil, adding 5 cc of a 10% $K_2SO_4$ soln and 10 cc of a 10% Ba-acetate soln. Collect the precipitate in a compact mass (a centrifuge may be used advantageously) and wash upon a small filter. Transfer to a Pt crucible and ignite.

Dip a carefully cleaned glass plate, while hot, in a mixture of equal parts of carnauba wax and paraffin and allow to cool. Make a distinctive mark thru the wax with a sharp instrument, taking care not to scratch the surface of the glass.

Add a few drops of $H_2SO_4$ to the residue in the crucible and cover the crucible with the waxed plate, having the mark over the center of the crucible and making sure that the edge of the crucible is in close contact with the plate. Keep the top surface of the plate cool by means of a suitable device and heat the crucible for an hour at as high a temp. as practicable without melting the wax (an electric stove gives the most satisfactory form of heat). If fluorides are present, a distinct etching will be apparent on the glass where it was exposed.

### 26 QUALITATIVE TEST II—OFFICIAL

(Applicable in the presence of silicates)

Test I may be varied by mixing a small quantity of precipitated $SiO_2$ with the precipitated $BaF_2$ and applying the method for the detection of fluosilicates, **28** or **29**.

This method is of value in the case of foods the ash of which contains a considerable quantity of $SiO_2$. Under these circumstances $H_2SO_4$ liberates $SiF_4$ which would escape detection under **25**.

## INSOLUBLE FLUORIDES

(Fluoborates, Fluosilicates, Etc.)

### 27 PREPARATION OF SAMPLE—OFFICIAL

Make about 200 g of the sample alkaline with lime $H_2O$, evaporate to dryness, and incinerate. Extract the crude ash with $H_2O$, to which has been added sufficient acetic acid to decompose carbonates; filter; ignite the insoluble portion; extract with acetic acid (1+2); and again filter. The insoluble portion now contains $CaSiO_3$ and $CaF_2$, while the filtrate contains all the $H_3BO_3$ present.

### 28 QUALITATIVE TEST I[10]—OFFICIAL

Incinerate the filter containing the insoluble portion from **27**, mix with a little precipitated $SiO_2$ transfer to a short test tube attached to a small U-tube containing a few drops of $H_2O$, and add 1–2 cc of $H_2SO_4$. Keep the test tube in a beaker of $H_2O$ on a steam bath for 30–40 min. If any F is present, the $SiF_4$ generated will be decomposed by the $H_2O$ in the U-tube and will form a gelatinous deposit on the walls of the tube.

Next test the filtrate for boric acid as directed under **15**. If both HF and $H_3BO_3$ are present, it is probable that they are combined as $BF_3$. If, however, $SiF_4$ is detected and $H_3BO_3$ is not, repeat the test without introducing the $SiO_2$, in which case the formation of the silica skeleton is conclusive evidence of the presence of fluosilicate. In an ash containing an appreciable quantity of $SiO_2$, $H_2SO_4$ will liberate $SiF_4$ rather than HF. Therefore the presence of a fluosilicate, not a fluoride, is indicated.

### 29 QUALITATIVE TEST II—OFFICIAL

Incinerate the filter containing the insoluble portion from **27** in a Pt crucible, mix with a little precipitated $SiO_2$, and add 1 cc of $H_2SO_4$. Cover the crucible with a watch-glass from the underside of which a drop of $H_2O$ is suspended, and heat for a n hour at 70–80°, keeping the watch-glass well cooled. The $H_2O$ decomposes the $SiF_4$ which is formed, leaving a gelatinous deposit of $SiO_2$ and etching a ring at the periphery of the drop of $H_2O$. Test the filtrate for $H_3BO_3$ as directed under **15**.

## SULFUROUS ACID

### 30 QUALITATIVE TEST[11]—OFFICIAL

Add a small quantity of S-free zinc and several cc of HCl to about 25 g of the sample (with the addition of $H_2O$, if necessary) in a 200 cc Erlenmeyer flask. The $H_2S$ generated in the presence of sulfites may be detected with Pb-acetate paper. The traces of metallic sulfides occasionally present in vegetables will give the same reaction as sulfites under the conditions of the above test. Verify positive results obtained by this method by the distillation method, **31**.

It is always advisable to make the quantitative determination of sulfites, owing to the danger that the test may be caused by traces of sulfides. A trace should not be considered sufficient indication of the presence of $SO_2$ either as a bleaching agent or as a preservative.

### TOTAL SULFUROUS ACID

### 31 *Distillation Method—Official*

(Not applicable if volatile organic sulfur compounds may be set free)

After adding about 5 cc of a 20% $H_3PO_4$ soln to 20–100 g of the sample, distil (adding recently boiled $H_2O$ if necessary) in a current of $CO_2$ until 150 cc has passed over. Collect the distillate in about 100 cc of nearly saturated Br water, allowing the end of the condenser to dip below the surface. The method and apparatus may be simplified without material loss in accuracy by omitting the current of $CO_2$, adding 10 cc of the $H_3PO_4$ instead of 5 cc, and dropping into the distillation flask, immediately before attaching the condenser, a piece of $NaHCO_3$ weighing not more than 1 g. The $CO_2$ liberated is not sufficient to expel all the air from the apparatus, but it will prevent oxidation to a large extent. When the distillation is finished, boil off the excess of Br, dilute the soln to about 250 cc, add 5 cc of HCl (1+3), heat to boiling, and precipitate the $H_2SO_4$ with $BaCl_2$ soln (approximately 10%). Boil for a few minutes longer, allow to stand overnight in a warm place, filter thru a weighed Gooch crucible or thru an ashless filter paper, wash with hot $H_2O$, ignite at a dull red heat, and weigh as $BaSO_4$. Conduct a blank determination on the apparatus and reagents used and correct the result accordingly.

### 32 *Direct Titration Method—Official*

In the examination of white grape juice, wine, and similar products, fairly accurate results may be obtained by the following method:

Place 25 cc of a 1 *N* NaOH or KOH soln in a 200 cc flask, add 50 cc of the sample, mix, and allow to stand for 15 min. with occasional agitation. Add 10 cc of $H_2SO_4$ (1+3) and a few cc of starch indicator [**VI, 3** (e)], and titrate with 0.02 *N* I soln. Introduce the I soln as rapidly as possible and continue the addition until the blue color persists for several min. 1 cc of 0.02 *N* I = 0.64 mg of $SO_2$.

### 33 FREE SULFUROUS ACID—OFFICIAL

(Especially adapted to wine)

Treat 50 cc of the sample in a 200 cc flask with about 5 cc of $H_2SO_4$ (1+3), add about 0.5 g of $Na_2CO_3$ to expel the air, and titrate the sulfurous acid with 0.02 *N* I, as directed under **32**.

### 34 *Monier-Williams Method*[12]*—Tentative*

Connect a 750 cc round-bottomed Pyrex flask (B) (Fig. 34) to a sloping reflux condenser (D), the lower end of which is cut off at an angle. (Monier-Williams prefers using an upright round-bottomed flask with 2 necks.) Pass $CO_2$ from a generator thru a $Na_2CO_3$ soln in A to remove Cl. Also connect a dropping funnel (K) to B by the three-hole stopper C. Use the tube E to connect the upper end of the condenser to a 200 cc Erlenmeyer flask (F), which is followed by a Peligot tube (G). This delivery tube (E) extends to the bottom of the receiver. One Peligot tube has been found to be sufficient to catch traces of sulfurous acid swept thru the flask F. Use rubber stoppers thruout. The receiver F contains 15 cc of pure neutral 3% $H_2O_2$, while the Peligot tube contains 5 cc. $H_2O_2$ usually contains free $H_2SO_4$. Start with 30% $H_2O_2$, dilute somewhat, and neutralize with $Ba(OH)_2$ soln, using bromphenol blue soln as indicator. After the reagent has settled in the cold, filter from the $BaSO_4$, determine its strength by permanganate titration, and finally adjust to a 3% strength. The bromphenol blue indicator in the $H_2O_2$ remains unaffected for some time.

After connecting the apparatus, introduce into the flask 300 cc of distilled $H_2O$ and 20 cc of HCl and boil for a short time in a current of $CO_2$. Then add the food to be tested, adopting the procedure to the sort of food. Add liquids directly by means of the dropping funnel. In the case of easily transferable solids, first cool the contents of the flask somewhat, taking care to avoid having the $H_2O_2$ drawn up in the delivery tube E by regulating the flow of $CO_2$. Then quickly introduce the food by removing the stopper C. With semi-solid foods, requiring more time to introduce into the flask, cool the flask contents by gradual immersion in cold $H_2O$, and wash the food in quickly with recently boiled distilled $H_2O$. After introducing the food, boil the mixture for 1 hour in a slow current of $CO_2$, stopping the flow of $H_2O$ in the condenser just before the end of the distillation. This causes the condenser to become hot and drives over residual traces of $SO_2$ retained in the condenser. When the delivery tube just above the receiver E becomes hot to the touch, remove stopper J immediately.

Wash the delivery tube and the Peligot tube contents into the flask F, and titrate the liquid at room temp. with 0.1 *N* NaOH, using bromphenol blue as indicator. The NaOH must be standardized with this indicator. Bromphenol blue is unaffected by $CO_2$ and also gives a distinct color change in cold $H_2O_2$. 1 cc of 0.1 *N* NaOH = 3.2 mg of $SO_2$, so that titration of small quantities of $SO_2$, requiring less than 0.5 cc of NaOH is not accurate. A gravimetric determination may be made after titration, the precipitation of $BaSO_4$ being carried out at room temp. After settling, the supernatant liquid is filtered, and the residual $BaSO_4$ is washed 3 times by decantation with boiling $H_2O$. Determine a blank on the reagents, both by titration and gravimetrically, and correct the results accordingly.

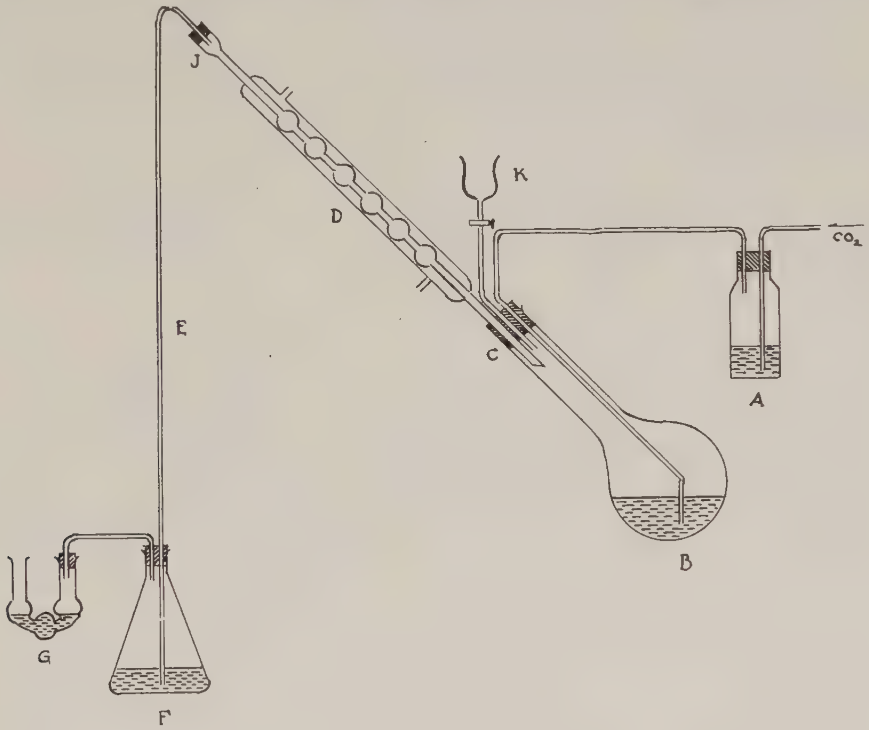

FIG. 34.—MONIER-WILLIAMS APPARATUS FOR DETERMINATION OF SULFUROUS ACID

## BETA-NAPHTHOL

**35** QUALITATIVE TEST—TENTATIVE

In a separatory funnel extract 200 cc of the sample or of its aqueous extract, prepared as directed under **1** (**c**), with 10 cc of $CHCl_3$. To the $CHCl_3$ extract in a test tube add a few drops of 0.5 *N* KOH soln and place in a boiling water bath for 2 min.

The presence of beta-naphthol is indicated by the formation of a deep blue color which changes to green and then to yellow.

## ABRASTOL (ASAPROL)

### 36 I. SINIBALDI METHOD[13]—TENTATIVE

Make 50 cc of the sample alkaline with a few drops of $NH_4OH$ and extract with 10 cc of amyl alcohol, adding ethyl alcohol if an emulsion forms. Decant the amyl alcohol, filter if turbid, and evaporate to dryness. Add to the residue 2 cc of $HNO_3$ (1+1), heat on a water bath until half of the liquid is evaporated, and transfer to a test tube with the addition of 1 cc of $H_2O$. Add about 0.2 g of $FeSO_4$ and an excess of $NH_4OH$, dropwise, with constant shaking. If the resultant precipitate is of a reddish color, dissolve it in a few drops of $H_2SO_4$ and add $FeSO_4$ and $NH_4OH$ as before. As soon as a dark colored or greenish precipitate is obtained introduce 5 cc of 95% alcohol, dissolve the precipitate in $H_2SO_4$, shake well, and filter. In the absence of abrastol this method gives a colorless or light yellow liquid, while a red color is produced in the presence of 0.01 g of abrastol.

### 37 II. SANGLÉ-FERRIÈRE METHOD[14]—TENTATIVE

Boil 200 cc of the sample with 8 cc of HCl for an hour in a flask fitted with a reflux condenser. Abrastol is thus converted into beta-naphthol and is detected as directed under **35**.

## SUCROL OR DULCIN

### 38 I. MORPURGO METHOD[15]—TENTATIVE

Evaporate about 100 cc of the sample or of the aqueous extract prepared as directed under **1 (c)** and neutralized with acetic acid to a sirupy consistency after the addition of about 5 g of $PbCO_3$, and extract the residue several times with 90% alcohol. Evaporate the alcoholic extract to dryness, extract the residue with ether, and allow the ether to evaporate spontaneously in a porcelain dish. Add 2 or 3 drops each of phenol and $H_2SO_4$ and heat for about 5 min. on a water bath. Cool, transfer to a test tube, and overlay with $NH_4OH$ or NaOH soln with the least possible mixing. The presence of dulcin is indicated by the formation of a blue color at the zone of contact.

### 39 II. JORISSEN METHOD[16]—TENTATIVE

Suspend the residue from the ether extract obtained as directed under **38** in about 5 cc of $H_2O$, add 2–4 cc of an approximately 10% soln of $Hg(NO_3)_2$, and heat for 5–10 min. on a steam bath. In the presence of sucrol a violet blue color is formed. On the addition of $PbO_2$ the color changes to a deep violet.

## FORMIC ACID[17]—OFFICIAL

### 40 REAGENTS

**(a)** *Sodium acetate.*—Dissolve 50 g of dry Na-acetate in sufficient $H_2O$ to make 100 cc and filter.

**(b)** *Mercuric chloride.*—Dissolve 100 g of $HgCl_2$ and 150 g of NaCl in sufficient $H_2O$ to make 1 liter and filter.

**(c)** *Hydrochloric acid.*—Approximately 10% soln. Dilute 30 cc of strong acid with 70 cc of $H_2O$.

### 41 APPARATUS

The apparatus required (Fig. 35) consists of a steam generator (S); a 300 cc flask (A) in which the sample is placed; a 500 cc flask (B), containing a suspension of

$BaCO_3$; a spray trap (T); a condenser; and a 1 liter volumetric flask (C). The tip of the tube (D), leading into (B), consists of a bulb containing a number of small holes to break the vapor into small bubbles.

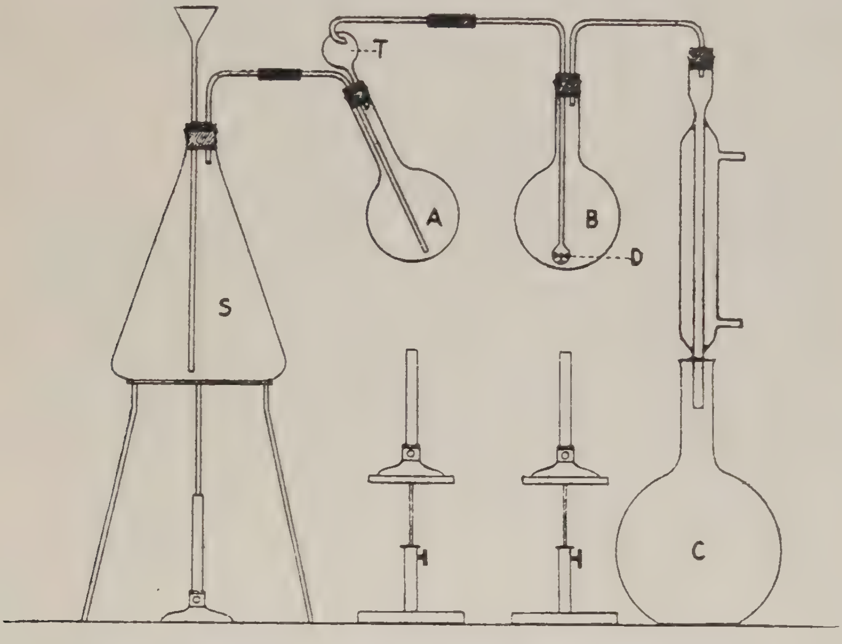

FIG. 35.—APPARATUS FOR DETERMINATION OF FORMIC ACID

**42** DETERMINATION

Use 50 cc of thin liquids like fruit juices; for heavy liquids and semi-solids like sirups and jams, use 50 g diluted with 50 cc of $H_2O$. Place the sample in flask A, add 1 g of tartaric acid, and connect as shown in Fig. 35, the flask B having been charged previously with a suspension of 2 g of $BaCO_3$ in 100 cc of $H_2O$. If much acetic acid is present, use sufficient $BaCO_3$ so that at least 1 g remains at the end of the operation. Heat the contents of flasks A and B to boiling and distil with steam from the generator S, the vapor passing first thru the sample in flask A, then thru the boiling suspension of $BaCO_3$ in B, after which it is condensed and collected in the volumetric flask C. Continue the distillation until 1 liter of distillate is collected, maintaining the volume of the liquids in the flasks A and B as nearly constant as possible by heating with small Bunsen flames and avoiding charring of the sample in the flask A. After 1 liter of distillate has been collected, disconnect the apparatus and filter the contents of flask B while hot, washing the $BaCO_3$ with a little hot $H_2O$. The filtrate and washings should now measure about 150 cc; if they do not, they should be boiled down to that volume. Add 10 cc of the Na-acetate soln, 2 cc of the dilute HCl, and 25 cc of the $HgCl_2$ reagent. Mix thoroly and immerse the container in a boiling water or steam bath for 2 hours. Filter thru a weighed Gooch crucible and wash the precipitate thoroly with cold $H_2O$ and finally with a little alcohol. Dry in a boiling water oven for 30 min., cool, weigh, and calculate the weight of HCOOH present by multiplying the weight of the precipitate by 0.0975. If the weight of $HgCl_2$ obtained exceeds 1.5 g, repeat the determination, using more $HgCl_2$ reagent or a smaller quantity of sample. Conduct a blank test with each new lot of reagents employed for the reduction, using 150 cc of $H_2O$, 1 cc of 10% $BaCl_2$ soln, 2 cc of the dilute HCl, 10 cc of the Na-acetate, and 25 cc of the $HgCl_2$ reagent, and heat the mixture in a boiling water or steam bath for 2 hours. Deduct the weight of HgCl obtained in this blank test from that obtained in the regular determination.

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# XXXIII. SPICES AND OTHER CONDIMENTS

## SPICES

### 1 PREPARATION OF SAMPLE—OFFICIAL

Grind the sample to pass thru a sieve having circular openings 1 mm in diameter and mix thoroly. Owing to the lack of uniformity of most spices and the peculiar tendency to stratify, use extreme care in weighing out a portion for analysis. Stir the material thoroly and weigh out a 2 g sample, using a spoon with a capacity of about 2 g. Dip a spoonful from the center of the material, being careful to take approximately the required quantity so as to avoid adding to or taking from the portion on the scale pan. In the determination of starch in spices by the diastase method, further reduce a subsample as nearly as possible to an impalpable powder.

### 2 MOISTURE—TENTATIVE

Dry 2 g to constant weight at 110°. From the resulting loss in weight subtract the quantity of volatile ether extract as determined under **9**.

### 3 ASH—OFFICIAL

Proceed as directed under **XXVII, 8.**

### 4 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 12,** using the ash obtained under **3.**

### 5 ASH INSOLUBLE IN ACID—OFFICIAL

Boil the water-insoluble residue obtained under **4,** or the total ash obtained as directed under **3,** with 25 cc of HCl (1+2.5) for 5 min., collect the insoluble matter on a Gooch crucible or on an ashless filter, wash with hot $H_2O$, ignite, cool, and weigh.

### 6 CALCIUM OXIDE IN ASH—OFFICIAL

Ignite 2–4 g of the sample as directed under **3,** digest with hot HCl (1+2.5), evaporate to dryness, moisten the dry residue with the dilute HCl, and again evaporate to dryness to render the $SiO_2$ insoluble. Treat the residue with 5–10 cc of HCl, add about 50 cc of $H_2O$, allow to stand on a water bath for a few min., filter, and wash the insoluble residue with hot $H_2O$. Determine CaO in the combined filtrate and washings as directed under **XII, 9.**

### 7 NITROGEN—OFFICIAL

Proceed as directed under **II, 19, 22** or **24,** except in the case of black and white peppers, for which use only the Kjeldahl-Gunning-Arnold method[1] (**II, 24**), using 1 g of the sample.

### 8 NITROGEN IN NON-VOLATILE ETHER EXTRACT—OFFICIAL

(For black and white peppers)

Extract 10 g of the pepper for 20 hours in a continuous extraction apparatus with absolute ether, collecting the extract in a weighed 250 cc flask. Evaporate the ether and dry first at 100° and finally to constant weight at 110°. Determine the N in the weighed extract as directed under **II, 24,** digesting in the same flask used for the extraction. Calculate the parts of N per 100 parts of non-volatile ether extract. If desired, crude piperine may be calculated from the N by multiplying by 20.36.

### 9 VOLATILE AND NON-VOLATILE ETHER EXTRACT[2]—OFFICIAL

Extract 2 g of the ground material for 20 hours in a continuous extraction apparatus with anhydrous ether (**XXVII, 15**). Transfer the ethereal soln to a weighed capsule and allow to evaporate at room temp. Let stand for 18 hours over $H_2SO_4$ and weigh the total ether extract. Heat the extract gradually and then to constant weight at 110°. The loss is volatile ether extract; the residue is non-volatile ether extract.

### 10 ALCOHOL EXTRACT[3]—OFFICIAL

Place 2 g of the sample in a 100 cc flask and fill to the mark with 95% alcohol. Stopper, shake for 8 hours at 30 min. intervals, and allow to stand for 16 hours longer without shaking. Filter the extract thru a dry filter, evaporate a 50 cc aliquot of the filtrate to dryness in a flat-bottomed dish on a steam bath, and heat to constant weight at 110°.

### 11 COLD-WATER EXTRACT—TENTATIVE

(For ginger)

Place 4 g of the sample in a 200 cc volumetric flask, add $H_2O$ to the mark, shake at 30 min. intervals during 8 hours, and let stand 16 hours longer without shaking. Filter and evaporate a 50 cc aliquot of the filtrate to dryness in a flat-bottomed metal dish. Dry to constant weight at 100°.

### 12 COPPER-REDUCING SUBSTANCES BY DIRECT INVERSION—OFFICIAL

Extract 4 g of the sample with 5 successive portions of 10 cc of ether on a filter that will retain completely the smallest starch granules. After the ether has evaporated, wash with 150 cc of alcohol, 10% by volume.

Owing to the formation of a glutinous mass, which clogs the filter, it is not possible to wash samples of Batavia cassia with $H_2O$ or dilute alcohol. Therefore it is best to omit all preliminary washing in determinations made on all varieties of cassia, as well as on cassia buds and cinnamon.

Carefully wash the residue from the paper into a 500 cc flask with 200 cc of $H_2O$, using a small wash bottle and gently rubbing the paper with the tip of the finger. Hydrolyze and determine the Cu reducing material as directed under **XXVII, 23.** Express the result in terms of starch.

### 13 STARCH—OFFICIAL

Extract 4 g of the finely pulverized sample with ether and 10% alcohol, as directed under **12**, and determine starch by the diastase method, as directed under **XXVII, 25.**

### 14 CRUDE FIBER—OFFICIAL

Proceed as directed under **XXVII, 19,** and previous to weighing remove all ether extractives by successive washings of the dry fiber with ether.

### 15 TANNIN—OFFICIAL

(For cloves and allspice)

Extract 2 g of the sample for 20 hours with anhydrous ether. Boil the residue for 2 hours with 300 cc of $H_2O$, cool, make up to 500 cc, and filter. Measure 25 cc of this infusion into a 1200 cc flask, add 20 cc of indigo soln and 750 cc of $H_2O$, and proceed as directed under **XVII, 51.** 1 cc of 0.1 $N$ oxalic acid = 0.00623 g of quercitannic acid, or 0.0008 g of O absorbed.

### 16 TOTAL SULFUR—OFFICIAL

(For mustard)

Proceed as directed under **XII, 23.**

### 17 VOLATILE OIL IN MUSTARD SEED[4]—OFFICIAL

Place 5 g of the ground seed (No. 20 powder) in a 200 cc flask, add 100 cc of $H_2O$, stopper tightly, and macerate for 2 hours at about 37°. Then add 20 cc of 95% alcohol and distil about 60 cc into a 100 cc volumetric flask containing 10 cc of $NH_4OH$ (1+2), taking care that the end of the condenser dips below the surface of the soln. Add 20 cc of 0.1 $N$ $AgNO_3$ soln to the distillate, set aside overnight, heat to boiling on a water bath in order to agglomerate the $(Ag)_2S$, cool, make up to 100 cc with $H_2O$, and filter. Acidify 50 cc of the filtrate with about 5 cc of $HNO_3$ and titrate with 0.1 $N$ $NH_4CNS$, using 5 cc of 10% ferric ammonium sulfate soln as an indicator. 1 cc of 0.1 $N$ $AgNO_3$ consumed = 0.004956 g of allylisothiocyanate.

### IODINE NUMBER OF PAPRIKA OIL

(Indicative of presence of olive oil)

### 18 *Qualitative Test—Tentative*

Transfer 10 g of the well-mixed ground sample to a 200 cc glass-stoppered flask and add 100 cc of $CHCl_3$ from a pipet, rotating while adding the first 50 cc. Let stand 1 hour, shake, and filter thru a 12½ cm fluted filter. Pipet off successively two 10 cc portions, using the same pipet. Transfer one of the 10 cc portions to a weighed crystallizing dish, 50 x 35 mm, and evaporate the solvent by placing the dish on a steam bath. Dry the dish and contents at 100° for 1 hour, cool in air, and weigh. Use the weight obtained in calculating the I Number. Transfer the other 10 cc portion to a suitable glass-stoppered flask or bottle for the determination of the I Number, proceeding as directed under **XXXI, 18,** allowing 30 min. for the halogen absorption. Calculate the I Number of the ether extract. The I Number of pure paprika thus obtained should be not less than 125.

### MICROSCOPIC EXAMINATION—TENTATIVE

### 19 GENERAL

Adulterants of vegetable origin in spices are best detected by means of the microscope. A general knowledge of vegetable histology and the microscopic appearance of the spices and spice adulterants is essential. Some of the standard works[6] on these subjects are listed under the selected references.

### 20 REAGENTS

Of the numerous reagents employed in histological work the following are the most useful in spice examinations:

**(a)** *Glycerol soln.*—Mix equal volumes of glycerol and $H_2O$.
**(b)** *Absolute alcohol.*
**(c)** *Ether.*
**(d)** *Ammonium hydroxide.*—For the turmeric test, (1+10).

(e) *Ammoniacal copper soln ("Schweitzer's reagent").*—Add slowly a soln of $CuSO_4$ to a soln of NaOH, separate by filtration the precipitate $Cu(OH)_2$ that forms and wash it thoroly with $H_2O$. Dissolve the wet precipitate in $NH_4OH$ with the aid of heat, cool, and filter. Prepare immediately before use and keep in the dark.

(f) *Potassium hydroxide soln.*—Dissolve 5 g of KOH in $H_2O$ and dilute to 100 cc.

(g) *Chloral hydrate.*—Dissolve 8 parts by weight of the crystals in 5 parts of $H_2O$.

(h) *Acidified chloral hydrate-glycerol soln.*—Dissolve 45 g of crystals of chloral hydrate in 25 cc of HCl (1+8) and 10 cc of glycerol.

(i) *Schultze's mixture.*—Mix crystallized $KClO_3$ with $HNO_3$ as needed.

(j) *Iodine-potassium iodide soln (iodine soln).*—Dissolve 0.05 g of I and 0.2 g of KI in 15 cc of $H_2O$.

(k) *Chlor-zinc iodine soln.*—Dissolve 100 g of $ZnCl_2$ in 60 cc of $H_2O$ in a glass-stoppered bottle and add 20 g of KI and 0.5 g of I crystals. A few crystals of I should be left in the bottle to insure saturation, and the soln should be allowed to stand a few hours before using. The chlor-zinc iodine soln prepared in this manner will keep for months. If the color developed in the tissue is too deep a blue, a very slight dilution of the reagent is advisable.

(l) *Millon's reagent.*—Prepare as directed under **XX, 15.**

(m) *Ferric acetate or chloride soln.*—Use a freshly prepared 1% aqueous soln.

(n) *Alkanet tincture.*—Macerate 20 g of alkanet root for several days with 100 cc of 95% alcohol.

(o) *Safranine soln.*—Prepare a saturated aqueous soln and dilute as needed.

(p) *Hydrochloric acid.*—Mix 1 volume of acid with 9 volumes of $H_2O$.

(q) *Acetic acid.*—Dilute 1 volume of glacial acetic acid with 2 volumes of $H_2O$.

(r) *Mayer's reagent (mercuric-potassium iodide soln).*—Dissolve 1.36 g of $HgCl_2$ in 60 cc of $H_2O$ and 5 g of KI in 10 cc of $H_2O$; mix these two solns and dilute to 100 cc.

## 21 APPARATUS

(a) *Dissecting microscope or hand lens.*

(b) *Compound microscope.*—Provided with objectives and oculars capable of giving a range of about 4 different magnifications varying from about 75 to 500 diameters, a double or triple nosepiece, and a substage condenser. An eyepiece micrometer, a polarizing apparatus, and a mechanical stage are desirable for some special types of work.

(c) *Sieves.*—A series of standard mesh sieves varying from 10 to 100 meshes per inch and a sieve having circular openings 1 mm in diameter.

(d) *Slides, cover glasses, needles, forceps, etc.*

## 22 PREPARATION OF SAMPLE

Reduce one portion to a fine powder in a mortar. Separate another portion into several grades of fineness by sieves of different mesh or by jarring on a sheet of paper. In the coarser grades fragments of suspicious nature may often be seen with the naked eye or under a simple microscope; these should be picked out for subsequent examination under the compound microscope.

## 23 EXAMINATION

Mount a small quantity of the ground sample in $H_2O$ and examine under the compound microscope with both ordinary and polarized light. This gives general information as to the nature of the material and serves for the detection and identification of starch granules and various tissues. Place a small drop of the I-KI soln at the edge of the cover-glass, draw it into the preparation by means of a piece of filter paper placed at the opposite edge of the cover-glass, and examine again. Starch granules will be colored blue or blue-black; cellulose, yellow; and proteins, either brown or yellow.

In the manner just described draw a little of the KOH soln under the cover-glass and again examine. This treatment gelatinizes the starch granules, dissolves the proteins, saponifies the fats, and in other ways clears the preparation. It also imparts to tannins a reddish color. If this treatment does not clear the tissues satisfactorily, treat a fresh portion for a short time with the acidified chloral hydrate-glycerol soln, or for some hours with the chloral hydrate soln.

Examine also the crude fiber obtained in the chemical analysis, as in this material the stone cells and other tissues are shown distinctly.

To isolate stone cells, bast fibers, and other thick-walled cells macerate a portion of the sample in Schultze's mixture, using such proportions of $KClO_3$ and $HNO_3$ and heating for such a time as will secure the desired results.

To distinguish cellulose from infiltrated substances (lignin, suberin, etc.), add freshly prepared chlor-zinc iodine soln to a water mount. Cellulose is colored blue, and infiltrated substances are yellow.

To distinguish fats, oils, essential oils, and resins from other cell contents, treat for an hour with alkanet tincture diluted with an equal volume of $H_2O$, which imparts to these substances a deep red color, or treat with ether, which dissolves them. Treat also with alcohol, which dissolves the essential oils and resins but does not perceptibly affect the fats and oils.

Test for proteins by warming cautiously on a slide with a drop of freshly prepared Millon's reagent. The proteins are partially decomposed, acquiring gradually a brick red color. If it is desired to study the form of the aleurone (protein) granules, which in some plants are quite as characteristic as starch granules, prepare a mount in pure glycerol or oil.

Test for tannins and tissues impregnated with them by adding 1% ferric acetate or chloride soln. Both of these reagents give a green or blue color with tannins, but the ferric acetate acts more slowly and is to be preferred.

Crystals of Ca-oxalate[7] are recognized by their characteristic forms and by their behavior to polarized light. To distinguish Ca-oxalate from $CaCO_3$, treat with acetic acid, which does not affect the oxalate but dissolves the carbonate with effervescence. Both are soluble in HCl.

Powdered charcoal and charred shells resist the bleaching action of potash, chloral hydrate, and Schultze's mixture.

## PREPARED MUSTARD

### 24 PREPARATION OF SAMPLE—OFFICIAL

Transfer the entire contents of the container to a dish sufficiently large to permit thoro stirring and make the whole mass homogeneous. Preserve in a bottle having a tightly fitting glass stopper. Stir well each time before removing a portion for analysis.

### 25 SOLIDS—OFFICIAL

Weigh 5 g of the sample into a flat-bottomed, Pt dish; distribute evenly over the bottom of the dish with a little $H_2O$; place on a steam bath until the mixture appears dry; and then heat in an oven at 100° to constant weight.

### 26 ASH—OFFICIAL

Ignite the dry residue, obtained in the determination of solids, **25**, as directed under **XXVII, 8.**

### 27 SALT—OFFICIAL

Determine Cl in the ash as directed under **XII, 33** or **35.**

### 28 ETHER EXTRACT—TENTATIVE

Weigh 10 g of the sample into any ordinary Si, Al or porcelain drying dish and mix with about 30 g of sand. Heat on a water bath until the mixture appears dry and complete the drying in a water oven. Grind until all the lumps are broken up, and determine the ether extract as directed under **XXVII, 15.**

### 29 PROTEIN—OFFICIAL

Determine the N as directed under **II, 19, 22,** or **24,** using 5 g of the sample. Multiply the result by 6.25 to obtain the equivalent quantity of protein.

### 30 ACIDITY—OFFICIAL

Weigh 10 g of the sample into a 200 cc volumetric flask, dilute to the mark with $H_2O$, shake, filter thru a dry paper, and determine the acidity in 100 cc by titration with 0.1 *N* alkali, using phenolphthalein indicator. Express the result as acetic acid. 1 cc of 0.1 *N* alkali = 0.0060 g of acetic acid.

### 31 COPPER-REDUCING SUBSTANCES BY DIRECT INVERSION—OFFICIAL

Proceed as directed under **XXVII, 23,** except to treat directly 10 g of the sample, without previous washing or extraction, with 200 cc of $H_2O$ and 20 cc of HCl (sp. gr. 1.125) and to make up the soln to 250 cc after neutralizing and before filtering and drawing off the aliquot. In analyses of samples containing starch, particular attention should be given that the quantity of dextrose present in the aliquot taken for the reducing sugar determination does not exceed the maximum permitted for that determination. Express the result in terms of starch.

### 32 CRUDE FIBER[8]—OFFICIAL

Weigh 10 g of the sample and transfer to an 8-ounce nursing bottle with 50 cc of 95% alcohol, stopper, and shake vigorously. Add 40 cc of ether, shake, and let stand about 5 min. with occasional shaking. Centrifugalize and decant the alcohol-ether mixture. Treat twice more with 40 cc portions of ether, shaking, centrifugalzing, and decanting as before. Rest the bottle on its side for a short time, without heat, to allow most of the ether to evaporate. Transfer the material to a 500 cc Erlenmeyer flask, using the 200 cc of boiling $H_2SO_4$ [**XXVII, 17 (a)**], and proceed as directed under **XXVII, 19,** but in addition wash the fiber with successive portions of ether previous to drying and weighing.

If preferred, the sample may be treated with alcohol and ether in a small beaker, transferred to a hardened 11 cm filter paper, washed several times with ether, and transferred to the 500 cc Erlenmeyer flask with the 200 cc of boiling hot dilute $H_2SO_4$.

### 33 COLORING MATTER—TENTATIVE

Proceed as directed under **XXI.**

### 34 PRESERVATIVES—OFFICIAL

Proceed as directed under **XXXII.**

## SALAD DRESSINGS[9]

### 35 PREPARATION OF SAMPLE—TENTATIVE

Before removing any portion of the sample for analysis and each time a subsequent portion is removed, if the sample has stood for any appreciable length of time, mix until it is homogeneous. For the various determinations, take approximately the quantity directed and weigh. A Bailey weighing buret[10] will be found convenient.

### 36 TOTAL SOLIDS—TENTATIVE

Weigh 10 g of the sample into a weighed, soft-metal dish (bottle cap) approximately 60 mm in diameter and containing 10–15 g of clean, dry quartz sand weighed with the dish. Evaporate to apparent dryness on a steam bath and then dry to constant weight at the temp. of boiling $H_2O$ under a pressure of not to exceed 100 mm of Hg. Cool in a desiccator and weigh. Weighings on samples high in solids should be made at 1-hour intervals. Reserve the dry material for the determination of lecithin-$P_2O_5$, **43.**

### 37 REDUCING SUGARS BEFORE INVERSION—TENTATIVE

Weigh 20 g of the sample into a wide-mouthed, 4-ounce bottle and extract the oil by adding about 80 cc of petroleum ether, shaking, and centrifugalizing. Draw off as much as possible of the petroleum ether soln (conveniently done by using suction and a short-stemmed pipet), and repeat the treatment with petroleum ether until all the oil has been removed. This is indicated by the absence of color in the solvent. Usually about four extractions are required. Reserve the ether soln for identification of the oil. Remove the petroleum ether from the residue with a current of air and transfer the residue with $H_2O$ to a 100 cc volumetric flask. Add 5–10 cc of a fresh soln of $HPO_3$ (prepared by dissolving 5 g of the transparent lumps or sticks in cold $H_2O$ and diluting to 100 cc), mix thoroly, dilute to volume, and filter. Transfer 80 cc of the filtrate, or as large an aliquot as possible, to a 100 cc flask; neutralize with a strong soln of NaOH (1+1), using phenolphthalein indicator; cool; dilute to the mark; and determine reducing sugars on an aliquot as directed under **XXXIV, 35** Calculate to invert sugar.

Some dressings, particularly those containing starch, cannot be clarified by the above method. In such instances, transfer the residue from the oil determination, **41,** to a 250 cc flask with alcohol, 50% by volume, and proceed as directed under **XXVII, 20.**

### 38 REDUCING SUGARS AFTER INVERSION—TENTATIVE

Invert an aliquot of the soln under **37** as directed under **XXXIV, 23 (b)**, and determine the reducing sugars in the inverted soln as directed under **XXXIV, 35.** Calculate to invert sugar.

### 39 SUCROSE—TENTATIVE

Subtract the percentage of invert sugar obtained before inversion, **37,** from that obtained after inversion, **38,** and multiply the difference by 0.95.

### 40 TOTAL ACID—TENTATIVE

Dilute 10 g of the sample with 400–500 cc of recently boiled and cooled $H_2O$ and titrate with standard alkali, using phenolphthalein indicator. Calculate as acetic acid.

### 41 OIL—TENTATIVE

Weigh 2 g of the sample into a Röhrig tube or a similar apparatus, add 2 cc of strong $NH_4OH$, and mix thoroly. Add 10 cc of 95% alcohol and again mix. Then add sufficient $H_2O$ to fill the tube to the level of the outlet and proceed as directed under **XXII, 16,** beginning with "Add 25 cc of ether." Make at least 4 extractions and dry the oil to constant weight at 70° under a pressure of not to exceed 100 mm of Hg. (The residue remaining in the tube may be used for testing for turmeric.)

### 42 IDENTIFICATION OF OIL—TENTATIVE

Proceed as directed under **XXXI,** using the oil obtained by evaporating the petroleum ether extracts in the determination of reducing sugars, **37.**

### 43 LECITHIN-PHOSPHORIC ACID ($P_2O_5$)—TENTATIVE

Transfer the residue from the determination of total solids, **36,** to an extraction thimble. Cut the soft-metal dish used in the same determination into small pieces and also place in the extraction thimble. Extract with absolute alcohol, using an extractor of the siphon type in which the vapor heats the contents of the siphon thimble. (If such an extractor is not available, a Soxhlet extractor may be used by wrapping the section containing the thimble with paper to raise the temp. of the vapor surrounding the thimble.) Extract for 10 hours, transfer the soln to a porcelain or Pt dish, add 5 cc of alcoholic KOH soln (8 g of KOH per 100 cc of 95% alcohol) for each g of fat, evaporate to dryness on a steam bath, and char over asbestos. Treat the charred mass with $HNO_3$ (1+3), filter, and wash with $H_2O$. Return the residue with the paper to the dish and burn to a white ash. Treat again with the dilute $HNO_3$, filter, and wash. Determine the $P_2O_5$ in the combined filtrates as directed under **II, 7** or **10.**

### 44 EGG SOLIDS—TENTATIVE

Calculate the approximate quantity of egg solids present from the content of lecithin-$P_2O_5$ in the sample and in average sized eggs.

Juckenack[11] gives the following average values for the lecithin-$P_2O_5$ content of eggs.

| | WEIGHT OF MATERIAL | DRY SUBSTANCE | LECITHIN-$P_2O_5$ CONTENT |
|---|---|---|---|
| | *grams* | *grams* | *grams* |
| Yolk | 16 | 7.835 | 0.1316 |
| White | 31 | 4.540 | 0.0000 |
| Whole egg | 47 | 12.375 | 0.1316 |

## VINEGARS

(Unless otherwise noted, express results as grams per 100 cc)

### 45 PHYSICAL EXAMINATION—OFFICIAL

Note the appearance, color, odor, and taste.

### 46 PREPARATION OF SAMPLE—OFFICIAL

Mix thoroly and filter before proceeding with the analysis.

### 47 SPECIFIC GRAVITY*—OFFICIAL

Determine the specific gravity at 20/4° by means of a pycnometer, as directed under **XVII, 24.**

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* See note 9, p. xvii.

### 48 SOLIDS—OFFICIAL

Measure 10 cc of the sample into a weighed, flat-bottomed Pt dish having a bottom diameter of 50 mm; evaporate on a boiling water bath for 30 min.; and dry for exactly 2.5 hours in a water oven at the temp. of boiling $H_2O$. Cool in a desiccator and weigh. To obtain concordant results, it is necessary to use a dish of the size and shape stated and to dry for exactly the time specified.

### 49 ASH—OFFICIAL

(**a**) Measure 25 cc of the vinegar into a weighed Pt dish, evaporate to dryness on a steam bath, and proceed as directed under **XXVII, 8.**

(**b**) Evaporate 25 cc of the sample to dryness as directed under (**a**) and heat in a muffle at low heat to expel inflammable gases. Treat the charred portion with a few cc of $H_2O$, and evaporate to dryness on a steam bath; replace in the muffle at low redness for 15 min. and continue the alternate evaporation and heating until a white or gray ash is obtained; cool in a desiccator and weigh.

Useful information may often be obtained by noting the odor given off by the solids during charring.

### 50 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Treat the ash, obtained under **49**, as directed under **XXXIV, 12.**

### 51 ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under **XXXIV, 13,** using the soluble ash obtained under **50.** Express the result as the number of cc of *N* acid required to neutralize the soluble ash from 100 cc of the vinegar.

In case the relation of the ash and alkalinity of the soluble ash is abnormal, the composition of the ash should be studied, especially as to content of chlorides, sulfates, phosphates, and alkalies.[12]

### 52 SOLUBLE PHOSPHORIC ACID—OFFICIAL

Proceed as directed under **II, 7** or **10,** using the soln obtained under **51.** Express the result as mg of $P_2O_5$ in 100 cc of vinegar.

### 53 INSOLUBLE PHOSPHORIC ACID—OFFICIAL

Dissolve the water-insoluble ash obtained under **50** in approximately 50 cc of boiling $HNO_3$ $(1+8)$ and proceed as directed under **II, 7** or **10.** Express the result as mg of $P_2O_5$ in 100 cc of vinegar.

### 54 TOTAL ACIDS—OFFICIAL

Dilute 10 cc of the sample with recently boiled and cooled $H_2O$ until it appears very slightly colored and titrate with 0.5 *N* alkali, using phenolphthalein indicator. 1 cc of 0.5 *N* alkali = 0.030 g of acetic acid.

### 55 NON-VOLATILE ACIDS—OFFICIAL

Measure 10 cc of the vinegar into a 200 cc porcelain casserole, evaporate just to dryness, add 5–10 cc of $H_2O$, and again evaporate; repeat until at least 5 evaporations have been made. Add about 200 cc of recently boiled and cooled $H_2O$ and titrate with 0.1 *N* alkali soln, using phenolphthalein indicator. 1 cc of 0.1 *N* alkali = 0.0060 g of acetic acid.

### 56 VOLATILE ACIDS—OFFICIAL

To obtain the volatile acids subtract the quantity of non-volatile acids, **55**, from the quantity of total acids, **54**.

### 57 TOTAL REDUCING SUBSTANCES BEFORE INVERSION—OFFICIAL

Measure 25 cc of the sample into a 50 cc volumetric flask and add enough NaOH soln (1+1) nearly to neutralize the acid. Cool, dilute to the mark with $H_2O$, and determine the reducing substances in 20 cc of the soln as directed under **XXXIV, 38.** If the quantity of reducing substances is very small, use 40 cc of the soln. Calculate the result as invert sugar (for malt vinegar as dextrose).

### 58 TOTAL REDUCING SUBSTANCES AFTER INVERSION—OFFICIAL

Invert 25 cc of the sample in a 50 cc volumetric flask with 5 cc of dilute HCl, as directed under **XXXIV, 23** (**b**) or (**c**). Neutralize with NaOH soln (1+1) and determine reducing substances as directed under **57.**

### 59 NON-VOLATILE REDUCING SUBSTANCES (SUGAR)—OFFICIAL

(Useful in calculating the non-sugar solids)

Evaporate 50 cc of the sample on a steam or water bath to a sirupy consistency, add 10 cc of $H_2O$, and evaporate again. Repeat with 10 cc of $H_2O$. Transfer the residue to a 100 cc volumetric flask with about 50 cc of warm $H_2O$. Cool; invert with 10 cc of dilute HCl as directed under **XXXIV, 23** (**b**) or (**c**); nearly neutralize with NaOH soln (1+1); cool; dilute to the mark with $H_2O$; and determine reducing substances in 20 cc or 40 cc, depending on the quantity present, as directed under **XXXIV, 38.** Calculate the result as invert sugar (for malt vinegar as dextrose). If the results for total reducing substances before and after inversion show the absence of sucrose, the inversion is unnecessary and may be omitted.

### 60 VOLATILE REDUCING SUBSTANCES[13]—OFFICIAL

When sucrose is absent, subtract the quantity of non-volatile reducing substances, **59**, from the mean of the total reducing substances before inversion, **57**, and after inversion, **58**, when sucrose is present, subtract the quantity of non-volatile reducing substances, **59**, from the quantity of total reducing substances after inversion, **58.**

### 61 ALCOHOL—OFFICIAL

Measure 100 cc of the sample into a round-bottomed distillation flask. Make faintly alkaline with NaOH soln (1+1), distil almost 50 cc, dilute to 50 cc at the temp. of the sample, and determine the specific gravity at 20/4°* by means of a pycnometer. Obtain from Tables **19-21, XLII,** the percentage by volume or g per 100 cc, noting that the alcoholic strength of the distillate is twice that of the original vinegar. Undue foaming may be obviated by adding a small piece of paraffin, free from volatile constituents.

### GLYCEROL[14]—OFFICIAL

#### 62 REAGENTS

(**a**) *Strong potassium dichromate soln.*—Dissolve 74.55 g of dry, recrystallized $K_2Cr_2O_7$ in $H_2O$; add 150 cc of $H_2SO_4$; cool; and dilute with $H_2O$ to 1 liter at 20°. 1 cc of this soln = 0.01 g of glycerol. Owing to the high coefficient of expansion of this strong soln it is necessary to make all volumetric measurements of the soln at the same temp. as that at which it was diluted to volume.

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* See note 9, p. xvii

(b) *Dilute potassium dichromate soln.*—Measure 25 cc of the strong $K_2Cr_2O_7$ soln at 20° into a 500 cc volumetric flask and dilute to the mark with $H_2O$ at room temp. 20 cc of this soln = 1 cc of (a).

(c) *Ferrous ammonium sulfate soln.*—Dissolve 30 g of crystallized ferrous ammonium sulfate in $H_2O$, add 50 cc of $H_2SO_4$, cool, and dilute with $H_2O$ to 1 liter at room temp. 1 cc of this soln = approximately 1 cc of (b). As its value changes slightly from day to day, it must be standardized against (b) whenever used.

(d) *Potassium ferricyanide indicator.*—Dissolve 1 g of crystallized $K_3Fe(CN)_6$ in 50 cc of $H_2O$. This soln must be freshly prepared.

(e) *Milk of lime.*—Introduce 150 g of CaO, selected from clean hard lumps, prepared preferably from marble, into a large porcelain or iron dish; slake with $H_2O$, cool, and add sufficient $H_2O$ to make 1 liter.

(f) *Silver carbonate.*—Dissolve 0.1 g of $Ag_2SO_4$ in about 50 cc of $H_2O$, add an excess of $Na_2CO_3$ soln, allow the precipitate to settle, and wash with $H_2O$ several times by decantation until the washings are practically neutral. This reagent must be freshly prepared immediately before use.

### 63 DETERMINATION

Make evaporations on a water bath maintained at a temp. of 85–90°. The area of the dish exposed to the bath should not be greater in circumference than that covered by the liquid inside.

Evaporate 100 cc of the vinegar to 5 cc, add 20 cc of $H_2O$, and again evaporate to 5 cc to expel acetic acid. Treat the residue with about 5 g of 40-mesh sand and 15 cc of the milk of lime and evaporate almost to dryness, with frequent stirring, avoiding the formation of a dry crust or evaporation to complete dryness. Treat the moist residue with 5 cc of $H_2O$; rub into a homogeneous paste; add slowly 45 cc of absolute alcohol, washing down the sides of the dish to remove adhering paste; and stir thoroly. Heat the mixture on a water bath, with constant stirring, to incipient boiling; transfer to a suitable vessel; and centrifugalize. Decant the clear liquid into a porcelain dish and wash the residue with several small portions of hot alcohol, 90% by volume, by aid of the centrifuge. (If a centrifuge is not available, decant the liquid thru a folded filter into a porcelain dish. Wash the residue repeatedly with small portions of hot 90% alcohol, twice by decantation, and then by transferring all the material to the filter. Continue the washing until the filtrate amounts to 150 cc.) Evaporate to a sirupy consistency; add 10 cc of absolute alcohol to dissolve this residue; and transfer to a 50 cc glass-stoppered cylinder, washing the dish with successive small portions of absolute alcohol until the volume of the soln is 20 cc. Add 3 portions of 10 cc each of anhydrous ether, shaking thoroly after each addition. Let stand until clear, pour off thru a filter, and wash the cylinder and filter with a mixture of 2 volumes of absolute alcohol and 3 of anhydrous ether. If a heavy precipitate has formed in the cylinder, centrifugalize at low speed, decant the clear liquid, and wash 3 times with 20 cc portions of the alcohol-ether mixture, shaking the mixture thoroly each time and separating the precipitate by means of the centrifuge. Wash the paper with the alcohol-ether mixture and evaporate the filtrate and washings on the water bath to about 5 cc, add 20 cc of $H_2O$, and again evaporate to 5 cc; again add 20 cc of $H_2O$ and evaporate to 5 cc; finally add 10 cc of $H_2O$ and evaporate to 5 cc.

These evaporations are necessary to remove all the ether and alcohol, and when conducted at 85–90° they result in no loss of glycerol if the concentration of the latter is less than 50%.

Transfer the residue with hot $H_2O$ to a 50 cc volumetric flask, cool, add the $Ag_2CO_3$ prepared from 0.1 g of $Ag_2SO_4$, shake, and allow to stand 10 min. Then add 0.5 cc of basic Pb-acetate soln [**XXXIV, 18** (**a**)]; shake occasionally; and allow to stand 10 min. Make up to the mark, shake well, filter, rejecting the first portion of the filtrate, and pipet 25 cc of the clear filtrate into a 250 cc volumetric flask.

Add 1 cc of $H_2SO_4$ to precipitate the excess of Pb and then 30 cc of Reagent (**a**). Add carefully 24 cc of $H_2SO_4$, rotating the flask gently to mix the contents and avoid violent ebullition, and then place in a *boiling* water bath for exactly 20 min. Remove the flask from the bath, dilute, cool, and make up to the mark at room temp. The quantity of strong dichromate soln used must be sufficient to leave an excess of about 12.5 cc at the end of the oxidation, the quantity given above (30 cc) being sufficient for ordinary vinegar containing about 0.35 g or less of glycerol per 100 cc.

Standardize the ferrous ammonium sulfate soln against Reagent (**b**) by introducing from the respective burets approximately 20 cc of each of these solns into a beaker containing 100 cc of $H_2O$. Complete the titration, using Reagent (**d**) as an outside indicator. From this titration calculate the volume ($F$) of the ferrous ammonium sulfate soln equivalent to 20 cc of the dilute and therefore, to 1 cc of Reagent (**a**).

In place of Reagent (**b**) soln substitute a buret containing the oxidized glycerol with an excess of Reagent (**a**) and ascertain how many cc are equivalent to ($F$) cc of the ferrous ammonium sulfate soln and, therefore, to 1 cc of Reagent (**a**). Then 250, divided by this last equivalent, = the number of cc of Reagent (**a**) present in excess in the 250 cc flask after oxidation of the glycerol.

The number of cc of Reagent (**a**) added, minus the excess found after oxidation, multiplied by 0.02, gives the grams of glycerol per 100 cc of vinegar.

### 64 COLOR—OFFICIAL

Determine the depth of color in a Lovibond tintometer by good reflected daylight, using ½- or 1-inch cell and the brewer's scale. Report the result in terms of a ½-inch cell and so state.

### 65 COLOR REMOVED BY FULLERS' EARTH—TENTATIVE

To 50 cc of the sample add 10 g of fullers' earth, shake at intervals for 30 min., and filter thru a folded filter. Place as much of the filtrate as is available into a colorimeter tube and place an equal volume of the original sample in a corresponding tube; dilute both with $H_2O$ to a volume of 50 cc and compare colors. Express the result as percentage of color removed. Not all fullers' earth is satisfactory for this test. The efficiency of the reagent should be determined on a sample of distilled vinegar known to be colored with caramel.

### 66 POLARIZATION[15]—TENTATIVE

Whenever possible, polarize in a 200 mm tube without decolorizing. Report results on basis of 200 mm tube in degrees Ventzke. When necessary, decolorization may be accomplished as follows:

(**a**) To 50 cc of the sample add a measured quantity of saturated neutral Pb-acetate soln, avoiding an excess of Pb; filter; remove Pb with powdered anhydrous K-oxalate, and filter. Polarize and correct for the dilution with Pb-acetate soln.

(**b**) To 50 cc of the sample add decolorizing C, avoiding an excessive amount or length of treatment. Filter thru a double paper and polarize.

### 67 SULFATES—OFFICIAL, FIRST ACTION

To 100 cc of the absolutely clear sample, add 2 cc of approximately normal HCl;

heat to boiling; add 10 cc of hot $BaCl_2$ soln (1 g per 100 cc), dropwise; and continue the boiling 5 min., keeping the volume approximately constant by adding hot $H_2O$ from time to time as required. Allow to stand until the supernatant liquid is clear. Overnight is convenient, but this time should not be exceeded. Filter on an ashless filter paper or a weighed Munroe crucible.[16] Wash free from chlorides with hot $H_2O$, dry, ignite at a low red heat, cool, and weigh. Express the result as mg of $SO_3$ in 100 cc of vinegar.

### 68 FORMIC ACID[17]—OFFICIAL

Use the apparatus described under **XXXII, 41.** Introduce 100 cc of the sample into flask (*A*); add 0.4–0.5 g of tartaric acid; and place in position as shown in Fig 35, having previously charged the flask *B* with a suspension of 15 g of $CaCO_3$ in 100 cc of $H_2O$. Heat the contents of flasks *A* and *B* to boiling and distil with steam from the generator *S*, the vapor passing first thru the sample in flask *A* then thru the boiling suspension of $CaCO_3$ in flask *B*, after which it is condensed and measured in the receiver *C*. Maintain the volume of liquid in flask *B* as nearly constant as possible and reduce the volume of the sample in flask *A* to 30–40 cc by heating with small Bunsen flames. Continue the distillation until 1 liter of distillate is collected. Disconnect the apparatus, filter the $CaCO_3$ suspension, and wash the $CaCO_3$ that remains on the paper with a little hot $H_2O$. Render the filtrate faintly acid with HCl, add 10–15 cc of $HgCl_2$ reagent [**XXXII, 40 (b)**], mix, and heat on a boiling water bath for 2 hours. Filter on a weighed Gooch crucible and wash the precipitate thoroly with cold $H_2O$ and finally with a little alcohol. Dry in a boiling water oven for 30 min., cool in a desiccator, weigh, and calculate the weight of HCOOH present by multiplying the weight of the precipitate by 0.0975.

### 69 PENTOSANS—OFFICIAL*

## TARTARIC ACID AND TARTRATES

### 70 *Qualitative Test—Official*

Evaporate 50 cc of the vinegar in a porcelain dish to a volume of about 10 cc, filter into a test tube, add 1 cc of 25% $CaCl_2$ soln and 2 cc of 50% $NH_4$-acetate soln, and allow to stand overnight. In the presence of tartaric acid a deposit of Ca-tartrate is formed, the crystals of which may be identified under the microscope by their characteristic form.

### 71 TOTAL TARTARIC ACID—OFFICIAL

Evaporate 200 cc of the sample to a sirupy consistency to remove excess of acetic acid; dilute to the original volume with $H_2O$ in a volumetric flask, determine the acidity as directed under **54**, and determine total tartaric acid in a 100 cc aliquot as directed under **XVII, 48,** using 20 cc of alcohol in the precipitation instead of 15 cc.

## FREE MINERAL ACIDS

### 72 *I. Logwood Method[18]—Tentative*

Prepare an extract of logwood as follows: Pour 100 cc of boiling $H_2O$ upon 2 g of fresh logwood chips, allow the infusion to stand for a few hours, and filter. Place several drops of the liquid on a porcelain surface and dry on a water bath. Add to one of the spots a drop of the vinegar to be tested (after concentration if desirable) and evaporate to dryness. A yellow tint remains if free mineral acids are absent, a red tint if they are present.

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* See note 7, p. xvii.

### 73 *II. Methyl Violet Method—Tentative*

Add 5–10 cc of $H_2O$ to 5 cc of vinegar, and after mixing well add 4 or 5 drops of methyl violet soln (1 part of methyl violet 2B in 10,000 parts of $H_2O$). A blue or green coloration indicates the presence of a free mineral acid.

### 74 *III. Quantitative Method—Tentative*

To a measured quantity of the sample add a measured excess of standard alkali, evaporate to dryness, incinerate, and titrate the ash with standard acid, using methyl orange indicator. The difference between the number of cc of alkali first added and the number of cc of acid needed to titrate the ash represents the free mineral acid present.

### 75 METALS—TENTATIVE

Proceed as directed under **XXIX.**

### 76 DEXTRIN (QUALITATIVE TEST)—TENTATIVE

Evaporate 100 cc of the vinegar to a volume of about 15 cc. Add slowly and with constant stirring 200 cc of 95% alcohol and allow to stand overnight. The precipitate formed should be tested for dextrin by the optical rotation and color reaction with I.

### 77 SPICES AND ADDED PUNGENT MATERIALS (QUALITATIVE TEST)—TENTATIVE

Neutralize exactly a portion of the vinegar and test by taste and smell. Agitate the liquid with ether in a separatory funnel, remove, evaporate the ethereal layer, and note the odor and taste of the residue.

### 78 COLORING MATTERS—TENTATIVE

Proceed as directed under **XXI.**

### 79 PRESERVATIVES—OFFICIAL

Proceed as directed under **XXXII.**

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[11] Z. Nahr. Genussm., **3**, 11 (1900).

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[16] J. anal. Chem., **2**, 241 (1888); J. Am. Chem. Soc., **31**, 456, 928 (1909).

[17] Z. Nahr. Genussm., **21**, 1 (1911); **22**, 88 (1912).

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# XXXIV. SUGARS AND SUGAR PRODUCTS

## SUGARS, SIRUPS, AND MOLASSES

### 1 PREPARATION OF SAMPLE—OFFICIAL

(**a**) *Liquids* (*molasses, sirups, etc.*).—Mix materials thoroly. If crystals of sugar are present, dissolve them either by heating gently (avoiding loss of $H_2O$ by evaporation) or by weighing the whole mass, then adding $H_2O$, heating until completely dissolved and after cooling, reweighing. Calculate all results to the weight of the original substance.

(**b**) *Semi-solids* (*massecuites, etc.*).—Weigh 50 g of the sample into a 250 cc volumetric flask, treat with $H_2O$, fill to the mark, and mix thoroly; or weigh 50 g of the sample and dilute with $H_2O$ to 100 g. If insoluble material remains, mix uniformly by shaking before taking aliquots or weighed portions for the various determinations.

(**c**) *Solids* (*sugars, etc.*).—Grind, if necessary, and mix thoroly to secure uniform samples. In the case of raw sugars mix thoroly and in the shortest possible time on a glass plate with a spatula, reducing lumps when present with a glass or iron rolling pin; or mix thoroly and in the shortest possible time in a large, clean, dry mortar, using a pestle to reduce lumps when present.

## Moisture

## DRYING METHODS

### 2 SUGARS—OFFICIAL

Dry 2–5 g of the sample prepared as directed under **1**(**c**) in a flat dish (Ni, Pt, or Al) at the temp. of boiling $H_2O$ for 10 hours; cool in a desiccator and weigh. Dry again for an hour or until the change in weight is not more than 2 mg. In the case of sugars of large grain, heat at 105–110° to expel the last traces of occluded $H_2O$.[1] Report the loss in weight as moisture.

### MASSECUITES, MOLASSES, AND OTHER LIQUID AND SEMILIQUID PRODUCTS—OFFICIAL

### 3 *Drying upon Pumice Stone—Official*

Prepare pumice stone of 2 grades of fineness, one of which will pass thru a 1-mm sieve, the other thru a 6-mm but not a 1-mm sieve. Digest each with $H_2SO_4$ $(1+4)$ for 8 hours on a steam bath. Wash free from acid and heat to dull redness. Make the determination in a flat metallic dish 60 mm in diameter. Place a layer of the fine pumice stone, 3 mm in thickness, on the bottom of the dish, then a layer of the coarse pumice stone, 6–10 mm in thickness; dry; and weigh. Dilute the sample with a weighed portion of $H_2O$ so that the diluted material shall contain 20–30% of solid matter. Weigh into the dish, prepared as described, the quantity of diluted sample to yield, approximately, 1 g of dry matter. If this weighing can not be made rapidly, use a weighing bottle provided with a cork thru which a pipet passes. Dry at 70° under a pressure of not to exceed 100 mm of Hg, making trial weighings at intervals of 2 hours toward the end of the drying period until the change in weight does not exceed 2 mg. Report the loss in weight as moisture. For substances containing little or no levulose or other readily decomposable substance, the drying may be made in a water oven at the temp. of boiling $H_2O$.

### 4 *Drying upon Quartz Sand*[2]—*Official*

Digest pure quartz sand that will pass a 40-mesh but not a 60-mesh sieve with HCl, wash free from acid, dry, and ignite. Preserve in a stoppered bottle. Place 25–30 g of the prepared sand and a short stirring rod in a dish approximately 55 mm in diameter and 40 mm in depth, fitted with a cover. Dry thoroly, cover dish, cool in a desiccator, and weigh immediately. Then add sufficient diluted sample to yield approximately 1 g of dry matter and mix thoroly with the sand. Heat on a steam bath for 15–20 min., stirring at intervals of 2–3 min., or until the mass becomes too stiff to manipulate readily. Dry at 70° under a pressure of not to exceed 100 mm of Hg, making trial weighings at 2-hour intervals toward the end of the drying period until the change in weight does not exceed 2 mg.

For materials containing no levulose or other readily decomposable substance the material may be dried at atmospheric pressure in a water oven at the temp. of boiling $H_2O$, heated for 8–10 hours, cooled in a desiccator, and weighed, the heating and weighing being repeated until the loss in 1 hour does not exceed 2 mg. Report the loss in weight as moisture.

(Dry sand, as well as the dried sample will absorb an appreciable quantity of moisture on standing over most desiccating agents, so all weighings should be made as quickly as possible after cooling in the desiccator.)

## Solids

### DENSIMETRIC METHODS

(Not applicable to low-grade sugar products, molasses, and other materials containing large quantities of non-sugar solids.)

### 5 *By Means of a Spindle—Official*

The density of juices, sirups, etc., is conveniently determined by means of the Brix or Baumé hydrometer, preferably the former, as the graduations of the scale give close approximations to the percentages of total solids. A table for comparison of degrees Brix (a density scale indicating directly the percentage by weight of pure sucrose in pure solns), degrees Baumé (modulus 145), sp. gr. at 20/4°, and sp. gr. at 20/20° is given under **XLII, 2.**

Employ a spindle graduated in tenths and as limited as possible in the range of degrees recorded and a cylinder of sufficient diameter to permit the spindle to come to rest without touching the sides. Allow the soln to come as nearly as practicable to the same temp. as the air at the time of reading, and if this varies more than 1° from the temp. at which the spindle was graduated, 20°, apply a correction according to **XLII, 3.** Before taking the density of a juice, allow it to stand in the cylinder until all air bubbles have escaped and all fatty or waxy matters have come to the top and been skimmed off. (Air bubbles may be conveniently removed, especially in the case of solns of high density, by applying vacuum to the cylinder by means of a tube passing thru a stopper inserted in the top of the cylinder.) If the sample is too dense to determine the density directly, dilute a weighed portion with a weighed quantity of $H_2O$, or dissolve a weighed portion and dilute to a known volume with $H_2O$. In the first instance the percentage of total solids is calculated by the following formula: Percentage of solids in the undiluted material $= \frac{WS}{w}$, in which

$S$ = percentage of solids in the diluted material;
$W$ = weight of the diluted material; and
$w$ = weight of sample taken for dilution.

When the dilution is made to a definite volume, the following formula is to be used:

Percentage of solids in the undiluted material $=\frac{VDS}{w}$, in which

$V$ = volume of the diluted soln at a given temp.;
$D$ = sp. gr. of the diluted soln at the same temp.;
$S$ = percentage of solids in the diluted soln at the same temp.; and
$w$ = weight of the sample taken for dilution.

### 6 *By Means of a Pycnometer—Official*

(**a**) *Specific gravity at 20/4°* (in vacuo)*.—Determine the sp. gr. of the soln at 20/4° by means of a pycnometer as directed under **XVII, 24,** and ascertain the corresponding percentage by weight of sugar solids from **XLII, 5.** When the density of the substance is too high for a direct determination, dilute, and then calculate the sucrose content of the original material as directed under **5.**

(**b**) *Specific gravity at 20/20° (in vacuo)*.—Proceed as directed under (**a**), making the determination at 20/20° instead of 20°/4°. Ascertain the corresponding percentage by weight of sugar solids from **XLII, 2.**

(**c**) *Specific gravity at 20/20° (in air)*.—*Tentative.*—Proceed as directed under (**b**) except that weighings are not corrected to weights in vacuo. Ascertain the corresponding percentage by weight of sugar solids from Domke's table[3] (**XLII, 4**).

### 7 *Refractometric Method—Official*

(Applicable only to liquid samples containing no undissolved solids)

Determine the refractometer reading of the soln at 20° and obtain the corresponding percentage of dry substance from either the direct reading, if a sugar refractometer is used, or from Table **6, XLII,** if the instrument gives readings in terms of refractive index. If the refractometer reading is obtained at a temp. other than 20°, correct the result according to Table **7, XLII.** If the soln is too dark to be read in the instrument, dilute with a concentrated sugar soln. $H_2O$ should never be used for this purpose. Mix weighed quantities of the soln under examination and a soln of pure sugar of about the same strength, and obtain the quantity of dry substance in the former by the following formula:

$x=\frac{(A+B)\,C-BD}{A}$, in which

$x$ = percentage of dry substance to be found;
$A$ = weight in g of the material mixed with $B$;
$B$ = weight in g of pure sugar soln employed in the dilution;
$C$ = percentage of dry substance in the mixture $A$ and $B$ obtained from the refractive index; and
$D$ = percentage of dry substance in the pure sugar soln obtained from its refractive index.

## Ash—Official

### CARBONATED ASH

### 8 *Method I*

Heat 5–10 g of the sample in a 50–100 cc Pt dish at 100° until the $H_2O$ is expelled, add a few drops of pure olive oil, and heat slowly over a flame until swelling ceases.

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* See note 9, p. xvii.

Then place the dish in a muffle and heat at low redness until a white ash is obtained. Treat the residue with a little $(NH_4)_2CO_3$ soln, re-evaporate, and heat again in the muffle at a very dull red heat to constant weight.

### 9 *Method II*

Carbonize 5–10 g of the sample in a 50–100 cc Pt dish at a low heat and treat the charred mass with hot $H_2O$ to dissolve the soluble salts. (In the case of low-purity products the addition of a few drops of pure olive oil, as in **8**, may be desirable.) Filter thru an ashless filter, ignite filter and residue to a white ash, add the filtrate of soluble salts, evaporate to dryness, and ignite gently. Treat the residue with a little $(NH_4)_2CO_3$ soln, re-evaporate, and heat again in the muffle at a very dull red heat to constant weight.

### 10 SULFATED ASH

Weigh 5 g of the sample into a 50–100 cc Pt dish, add 0.5 cc of $H_2SO_4$, ignite gently until the sample is well carbonized, and then burn in a muffle at low redness. Cool, add a few drops more of $H_2SO_4$, heat until fully volatilized, cool, and weigh. Express the result as percentage of sulfated ash.

### 11 MINERAL CONSTITUENTS—OFFICIAL

Proceed as directed under **XII.**

### 12 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Ash the material as directed under **8** or **9**. Add $H_2O$ to the ash in the Pt dish, heat nearly to boiling, filter thru an ashless filter, and wash with hot $H_2O$ until the combined filtrate and washings measure about 60 cc. Return the filter and contents to the Pt dish, ignite carefully, cool, and weigh. Calculate the percentages of water-soluble and water-insoluble ash.

### 13 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Cool the filtrate from **12** and titrate with 0.1 $N$ HCl [**II, 17 (a)**], using methyl orange indicator [**VI, 3 (f)**]. Express the alkalinity in terms of the number of cc of normal acid per 100 g of sample.

### 14 ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Add an excess of 0.1 $N$ HCl (usually 10–15 cc) to the ignited insoluble ash in the Pt dish, under **12,** heat to boiling on an asbestos plate, cool, and titrate the excess of HCl with 0.1 $N$ NaOH, using methyl orange indicator. Express the alkalinity in terms of the number of cc of normal acid per 100 g of sample.

### 15 MINERAL ADULTERANTS IN THE ASH—TENTATIVE

In a large porcelain evaporating dish, mix 100 g of the sample with about 35 g of $H_2SO_4$ and evaporate to a sirupy consistency. Pass an electric current thru it while stirring by placing one Pt electrode in the bottom of the dish near one side and attaching the other to the lower end of the glass rod with which the contents are stirred. Begin with a current of about 1 ampere and gradually increase to 4. In 10–15 min. the mass is reduced to a fine dry char, which may be readily burned to a white ash in the original dish over a free flame or in a muffle.

This method[4] is preferred to the ordinary method of heating with $H_2SO_4$, especially in the case of molasses, because if properly manipulated the material comes quickly into the form of a very finely divided char or powder that is especially adapted for subsequent quick ignition.

If an electric current is not available, treat in a large porcelain dish 100 g of the saccharine soln, evaporated to a sirupy consistency, with sufficient $H_2SO_4$ to carbonize the mass thoroly and ignite in the usual manner.

The following adulterants may be present: salts of tin, used in molasses to bleach; mineral pigments, such as $PbCrO_4$ in yellow confectionery; oxide of iron, sometimes used to simulate the color of chocolate; and Cu. These elements may be detected by the usual qualitative tests.

## 16 Nitrogen—Official

Determine N in 5 g of the material as directed under **II, 19, 22** or **24,** using a larger quantity of the $H_2SO_4$ if necessary for complete digestion.

## Sucrose

## POLARIMETRIC METHODS

### 17 GENERAL DIRECTIONS FOR RAW SUGARS—OFFICIAL

(Rules of the International Commision for Unifying Methods of Sugar Analysis[5])

"In general all polarizations are to be made at 20°.

"The verification of the saccharimeter must also be made at 20°. For instruments using the Ventzke scale 26 g of pure dry sucrose, weighed in air with brass weights, dissolved in $H_2O$ so that 100 cc of soln is obtained at 20° and polarized in a room or cabinet, the temp. of which is also 20°, must give a saccharimeter reading of exactly 100.00. The temp. of the sugar soln during polarization must be kept constant at 20°."

According to the determination of Bates and Jackson[6] at the U. S. Bureau of Standards, the Ventzke scale saccharimeter reading for 26 g of pure dry sucrose under the above conditions is $99.88_5$. The Ventzke scale reading has been re-determined by Balch and Hill[7] and by Zerban, Gamble and Hardin,[7] who found values of 99.907° and 99.912°, respectively. The average value of these three independent observers is $99.90_5$.

"For countries where the mean temp. is higher than 20°, saccharimeters may be adjusted at 30° or any other suitable temp., under the conditions specified above, provided the sugar soln be diluted to final volume and polarized at this same temp.

"In determining the polarization of substances containing sugar employ only half-shade instruments."

The saccharimeter used may have either a single or double wedge and should be a half-shadow instrument with either double or triple field.

"During the observation keep the apparatus in a fixed position and so far removed from the source of light that the polarizing Nicol is not warmed.

"As sources of light, employ lamps which give a strong illumination, such as triple gas burner with metallic cylinder, lens, and reflector; gas lamps with Auer (Welsbach) burner; electric lamp; petroleum duplex lamp; or Na light. Whenever there is any irregularity in the sources of light such as that due to the convolutions of the filament in the case of electric light or to the meshes of the gauze in the case of the Welsbach light, place a thin ground-glass plate between the source of light and the polariscope so as to render the illumination uniform.

"Before and after each set of observations the chemist must satisfy himself of the correct adjustment of his saccharimeter by means of standardized quartz plates. He must also previously satisfy himself of the accuracy of his weights, polarization flasks, observation tubes, and cover-glasses. (Scratched cover-glasses must not be be used.) Make several readings and take the mean thereof, but no one reading may be neglected."

The quartz plates are standardized to read to the second decimal place, and by their use a quick and at the same time accurate test can be made. In using such plates for testing saccharimeters, it is necessary that the instrument, as well as the plate, be at 20° before making a reading. Different points of the scale, preferably 20°, 50°, 80°, and 100° (sugar scale), should be tested against the plates. The scale may also be standardized by means of a carefully calibrated telescopic control tube.[8] A new type of telescopic control tube of high accuracy has recently been described by Zerban, Gamble and Hardin.[7]

"In determining the polarization use the whole normal weight for 100 cc or a multiple thereof for any corresponding volume.

"As clarifying and decolorizing agents use basic acetate of lead * * *, alumina cream, or concentrated soln of alum. Boneblack and decolorizing powders are to be excluded."

Whenever reducing sugars are determined in the soln for polarizing, use only neutral Pb-acetate for clarification, as basic Pb-acetate causes precipitation of some of the reducing sugars. In addition to the clarifying agents mentioned, basic Pb-nitrate has been made official by the association.

"After bringing the soln exactly to the mark at the proper temp., and after wiping out the neck of the flask with filter paper, pour all the well-shaken clarified sugar soln on a rapidly acting, dry filter. Reject the first portion of the filtrate and use the remainder, which must be perfectly clear, for polarization."

Cover the funnel at the start of filtration. It is advisable to reject the first 25 cc that runs thru, and use the remainder for polarization. In no case should the whole soln or any part be returned to the filter. If cloudy after the 25 cc has been rejected, begin a new determination.

"Whenever white light is used in polarimetric determinations, the same must be filtered thru a soln of $K_2Cr_2O_7$ of such a concentration that the percentage content of the soln multiplied by the length of the column of the soln in centimeters is equal to nine."

This concentration must be doubled in polarizing carbohydrate materials of high rotation dispersion, such as commercial glucose, etc.

## 18 PREPARATION AND USE OF CLARIFYING REAGENTS—OFFICIAL

(a) *Basic lead acetate soln.*—Boil 430 g of neutral Pb-acetate, 130 g of litharge, and 1 liter of $H_2O$ for 30 min. Allow the mixture to cool and settle and then dilute the supernatant liquid to a sp. gr. of 1.25 with recently boiled $H_2O$. Solid basic Pb-acetate may be substituted for the normal salt and litharge in the preparation of the soln.

(b) *Alumina cream.*—Prepare a cold saturated soln of alum in $H_2O$. Add $NH_4OH$ with constant stirring until the soln is alkaline to litmus, allow the precipitate to settle, and wash by decantation with $H_2O$ until the wash $H_2O$ gives only a slight test for sulfates with $BaCl_2$ soln. Pour off the excess of $H_2O$ and store the residual cream in a stoppered bottle.

(c) *Dry basic lead acetate.*—Obtained as a dry powdered salt and should contain 72.8% of Pb, which corresponds to a composition of 3 Pb $(C_2H_3O_2)_2$ 2 PbO. Of this salt, about $\frac{1}{3}$ g is equivalent to 1 cc of the basic Pb-acetate soln described under (a). In making the clarification, add a small quantity of the dry salt to the sugar soln after completion to volume and shake; then add more salt and shake again, repeating the addition until precipitation is complete, but avoiding any excess. When molasses or any other substance producing a heavy precipitate is being clarified, add some dry, coarse sand to break up the pellets of basic Pb-acetate and precipitate. (Dry basic Pb-acetate can also be used in place of a soln of this salt in clarifying cane, sorghum, and beet products.)

(d) *Neutral lead acetate.*—Prepare a saturated soln of neutral Pb-acetate and add it to the sugar soln before completing to volume. Its use is imperative when determining the reducing sugars in the soln used for polarization.

(e) *Basic lead nitrate.*—(1) Dissolve 250 g of $Pb(NO_3)_2$ in $H_2O$ and make up to 500 cc. (2) Dissolve 25 g of NaOH in $H_2O$ and make up to 500 cc. In making the clarification, add equal quantities of (1) and (2) to the sugar soln, shake, and add more if complete precipitation has not occurred, but avoid an excess. Then complete the volume with $H_2O$. When this soln is used for clarification, the factor in the Clerget determination by acid becomes 143.5.

### 19 TEMPERATURE CORRECTIONS FOR THE POLARIZATION OF SUGARS[9]—TENTATIVE

(a) *Refined sugars.*—The polarizations of sugars testing 99 or above, when made at temp. other than 20°, may be calculated to polarizations at 20° by the following formula:

$P_{20}=p^t\ (1+0.0003\ (t-20))$, in which
$P^t$ = the polarization at the temp. read; and
$t$ = the temp. at which the polarization is read.

(b) *Raw cane sugars.*—The polarizations of raw cane sugars, when made at temps. other than 20°, may be calculated to polarizations at 20° by the following formula:

$P_{20}=p^t+0.0015\ (p^t-80)\ (t-20)$, in which
$p^t$ and $t$ are the same as in the formula under (a).

When the percentage of levulose in the sugar is known (which in the case of honeys and sugar cane products is approximately ½ the reducing sugars), the following formula should be used:

$P_{20}=p^t+0.0003S\ (t-20)-0.00812L\ (t-20)$, in which
$p^t$ and $t$ are the same as in the formula under (a);
$S$ = the percentage of sucrose; and
$L$ = the percentage of levulose.

These formulas give results agreeing closely with the polarizations obtained at 20° if the sugar is of average normal composition.

### 20 MUTAROTATION

Products, such as honey and commercial glucose, that contain dextrose or other reducing sugars in crystalline form or in soln at high density may exhibit the phenomenon of mutarotation under the conditions prevailing during analysis. Only the constant rotation should be employed in polarimetric methods. To obtain this, allow the soln prepared for polarization to stand overnight before making the reading. If it is desired to make the reading immediately, heat the neutral (pH approximately 7.0) soln to boiling for a few moments or add a few drops of $NH_4OH$ before completing to volume; or, if the soln has been made to volume, add dry $Na_2CO_3$ until just distinctly alkaline to litmus paper. (Do not allow the slightly alkaline solns to stand at such high temps. or for such lengths of time as to cause destruction of fructose.) Determine the completion of mutarotation by making readings at 15–30-min. intervals until these become constant.

## SUCROSE IN THE ABSENCE OF RAFFINOSE

### *I. By Polarization Before and After Inversion with Invertase*[10]*—Official*

**21** REAGENT

*Invertase soln.*—Commercial invertase preparations are available on the market.[11] If it is desired to prepare the soln in the laboratory, the procedure described under (1) may be used. In either case the preparation may be further purified and concentrated by the ultrafiltration method described under (3). Commercial preparations may also be purified by dialysis and then reconcentrated by evaporating in vacuo at a temp. not to exceed 40°.

(1) *Crude invertase soln.*—Mix yeast with $H_2O$ in the proportion of 10 pounds of compressed baker's yeast with 5 liters of $H_2O$. Add 2 liters of toluene and stir thoroly at frequent intervals during the first 24 hours. Allow to stand for 7 days with occasional stirring and filter by gravity thru large fluted papers. Mix the residue with 2 liters of $H_2O$, filter, and combine the filtrates. Purify[12] by adding 15 g of neutral Pb-acetate to each liter of extract and filtering on paper after all Pb-acetate has been dissolved. Complete the purification immediately by dialysis or by washing on the ultrafilter as directed under (3).

(2) *Collodion ultrafilter.*[13]—Dissolve 6 g of soluble (in alcohol and ether mixture) pyoxylin or nitro-cellulose such as Astoria's in a mixture of 50 cc of absolute alcohol and 50 cc of absolute ether by first adding the alcohol to the cotton, allowing the mixture to stand in a stoppered flask for 10 min., adding the ether, and shaking. Allow the soln to stand overnight, pour about 100 cc into a 2000 cc cylinder, and coat the entire inside surface of the cylinder with the collodion. Drain, and dry for 10 min. Fill with $H_2O$, let stand 10–15 min., pour out the $H_2O$, and remove the collodion sack. Test for leaks by filling with $H_2O$. Slit open longitudinally and cut out a circular piece about 7–8 inches in diameter. Cut the bottom from a 2 liter bottle or Erlenmeyer flask and grind the edge smooth. Place it upon the still moist collodion disk, fold the edge of the disk up around the bottle, and cement it thereto with collodion that contains an increased percentage of ether. Place 3 or 4 thicknesses of wet filter paper in an 8-inch Büchner funnel. Place the bottle with the collodion membrane upon the filter paper. Pour melted vaseline, to the depth of an inch, between the bottle and inside of the funnel. Provide the bottle with a small mechanical stirring device.

(3) *Washing and concentration of invertase soln by ultrafiltration.*—Filter 4 liters of the partially purified soln thru the ultrafilter, stirring continuously, until about 1 liter remains. Wash with distilled $H_2O$ introduced by means of a constant level device until the filtrate is colorless, 3 or 4 liters of wash $H_2O$ being required. During the entire process the invertase soln must be preserved with toluene or chinosol.

(4) *Activity of invertase soln.*—The following test for activity of the invertase soln is usually adequate: Dilute 1 cc of the invertase preparation to 200 cc. Transfer 10 g of sucrose (granulated sugar) to a sugar flask graduated at 100 cc and 110 cc, dissolve in about 75 cc of $H_2O$, add 2 drops of glacial acetic acid, and dilute to the 100 cc mark. To the 100 cc of sugar soln add 10 cc of the dilute invertase soln and mix thoroly and rapidly, noting the exact time at which the solns are mixed. At the termination of exactly 60 min. make a portion of the soln just distinctly alkaline to litmus paper with anhydrous $Na_2CO_3$ and polarize in a 200 mm tube at 20°. If the invertase soln is sufficiently active, the alkaline soln will polarize approximately 31° Ventzke without correcting for the dilution to 110 cc and the optical activity of the invertase soln.

If more exact information concerning the activity of the invertase preparation is desired, determine its velocity constant as follows: Dilute 1 cc of the invertase soln to 200 cc at 20°; place in a constant temp. bath at 20°; and when the soln has attained the latter temp., pipet 20 cc of it into a flask containing 200 cc of a sucrose soln (10 g per 100 cc concentration) that has been previously made distinctly acid to methyl red (corresponding to pH approximately 4.6[4]) by the addition of acetic acid and also brought to a temp. of 20° in the same bath. Mix thoroly and promptly and note the time at which the invertase soln was added. Keep the sucrose-invertase mixture in the constant temp. bath; remove portions at the end of 15, 30, and 45 min.; render each portion just distinctly alkaline to litmus paper with anhydrous $Na_2CO_3$ immediately after removing; and polarize at 20°. Correct all polarizations for the polarization of the invertase soln. Calculate the velocity constant, $k$, for each of the polarizations (at the time $t$) subsequent to the initial polarization by the following formula:

$$k=\frac{\log_{10} 1.32\ \mathrm{R}_0-\log_{10}\ (R_t+0.32\ R_0)}{t}, \text{ in which}$$

$k$ = the unimolecular reaction velocity constant;

$t$ = number of min. elapsing from the time the invertase and sucrose solns were mixed until inversion was stopped by addition of $Na_2CO_3$.

$R_0$ = initial polarization (calculated by multiplying the polarization of the sucrose soln by 10/11 and correcting for the polarization of the invertase soln); and

$R_t$ = polarization at time $t$.

An invertase soln of sufficient activity[11] should yield an average value for $k$ (for the various time periods) of at least 0.1 after multiplying the $k$ value directly obtained by 200, in order to correct for the initial dilution of the invertase soln. The dilution of the invertase soln above mentioned is made solely for the purpose of determining its activity; the original, undiluted invertase soln is used as the inverting reagent in the determination of sucrose (**22**) unless the activity of the original invertase soln greatly exceeds a $K$ value of 0.1, and it is desirable to conserve the invertase. In this case, dilute to a $K$ value of 0.1, which is done in the same manner as diluting other solns to a standard strength. The activity of the invertase preparation required for rapid inversion, **22** (**c**), is the same as that needed for overnight inversion, **22** (**b**), but the proportion of invertase preparation used in the former case is twice that used in the latter instance.

## 22 DETERMINATION

(**a**) *Direct reading.*—Dissolve the double normal weight (52 g) of the substance in $H_2O$ in a 200 cc flask; add basic lead acetate soln carefully, avoiding any excess, then 1–2 cc of alumina cream; shake; dilute to the mark with $H_2O$; mix well; and filter, keeping the funnel covered with a watch-glass. Reject the first 25 cc of the filtrate. When sufficient filtrate has collected, remove the Pb from the soln by adding anhydrous $Na_2CO_3$, a little at a time, avoiding any excess; mix well; and filter again, rejecting at least the first 25 cc of the filtrate. (Instead of weighing 52 g into a 200 cc flask, two 26-g portions may be diluted to 100 cc each, and treated exactly as described. Depending on the color of the product, multiples or fractions of the normal weight may be used, and the results reduced by calculation to the basis of 26 g in 100 cc.) Pipet one 50 cc portion of the Pb-free filtrate into a 100 cc flask, dilute with $H_2O$ to the mark, mix well, and polarize in a 200 mm tube. The result, multiplied by 2, is the direct reading ($P$ of formula given below) or polarization before inversion. (If a 400 mm tube is used, the reading equals $P$.) If there is a possibility of mutarotation, proceed as directed under **20**.

**(b)** *Invert reading.*—First determine the quantity of acetic acid necessary to render 50 cc of the Pb-free filtrate distinctly acid to methyl red indicator [cf. **21** (4)]; then to another 50 cc of the lead-free soln in a 100 cc volumetric flask, add the requisite quantity of acid and 5 cc of the invertase preparation, fill the flask with $H_2O$ nearly to 100 cc, and let stand overnight (preferably at a temp. not less than 20°). Cool, and dilute to 100 cc at 20°. Mix well and polarize at 20° in a 200 mm tube. If the analyst is in doubt as to the completion of the hydrolysis, allow a portion of the soln to remain for several hours and again polarize. If there is no change from the previous reading, the inversion is complete, and the reading and temp. of the soln should be carefully noted. If it is necessary to work at a temp. other than 20°, which is permissible within narrow limits, the volumes must be completed and both direct and invert readings must be made at the same temp. Correct the invert reading for the optical activity of the invertase soln and multiply by 2. Calculate the percentage of sucrose by the following formula:

$$S=\frac{100\ (P-I)}{142.1+0.073\ (m-13)-t/2},\text{ in which}$$

$S$ = percentage of sucrose;
$P$ = direct reading, normal soln;
$I$ = invert reading, normal soln;
$t$ = temp. at which readings are made; and
$m$ = g of total solids in 100 cc of the invert soln read in the polariscope.

Determine the total solids as percentage by weight, as directed under **7**, and multiply this figure by the density at 20°, as obtained from **XLII, 5.**

**(c)** *Rapid inversion at 55–60°.*[16]—If more rapid inversion is desired, proceed as follows: Prepare the sample as directed under **(a)** and to 50 cc of the Pb-free filtrate in a 100 cc volumetric flask add glacial acetic acid in sufficient quantity to render the soln distinctly acid to methyl red [cf. **21**, (4)]. The quantity of acetic acid required should be determined before pipetting the 50 cc portion, as described under **22 (b)**. Then add 10 cc of invertase soln, mix thoroly, place the flask in a water bath at 55–60°, and allow to stand at that temp. for 15 min. with occasional shaking. Cool, add $Na_2CO_3$ soln until distinctly alkaline to litmus paper, dilute to 100 cc at 20°, mix well, and determine the polarization at 20° in a 200 mm tube. Allow the soln to remain in the tube for 10 min. and again determine the polarization. If there is no change from the previous reading, the mutarotation is complete. Carefully note the reading and the temp. of the soln. Correct the polarization for the optical activity of the invertase soln and multiply by 2. Calculate the percentage of sucrose by the formula given under **(b)**.

(If the soln has been rendered so alkaline as to cause destruction of sugar, the polarization, if negative, will in general decrease, since the decomposition of fructose ordinarily is more rapid than that of the other sugars present. If the soln has not been made sufficiently alkaline to complete mutarotation quickly, the polarization, if negative, will in general increase. As the analyst gains experience he may omit the polarization after 10 min. if he has satisfied himself that he is adding $Na_2CO_3$ in sufficient amount to complete mutarotation at once without causing any destruction of sugar during the period intervening before polarization.)

### **23** *II. By Polarization Before and After Inversion With Hydrochloric Acid*[17]—*Official*

(In the presence of much levulose, as in honeys, fruit products, sorghum sirup, cane sirup, and molasses, the optical method for sucrose, requiring hydrolysis by acid, gives erroneous results.)

(**a**) *Direct reading*.—Proceed as directed under **22** (**a**).

(**b**) *Invert reading*.—Pipet a 50 cc portion of the Pb-free filtrate into a 100 cc flask and add 25 cc of $H_2O$. Then add, little by little, while rotating the flask, 10 cc of HCl (sp. gr. 1.1029 at 20/4° (or 24.85° Brix at 20°)). Heat a water bath to 70° and regulate the burner so that the temp. of the bath remains approximately at that point. Place the flask in the water bath, insert a thermometer, and heat with constant agitation until the thermometer in the flask indicates 67°. This preliminary heating period should require from $2\frac{1}{2}$–$2\frac{3}{4}$ min. From the moment the thermometer in the flask indicates 67°, leave the flask in the bath for exactly 5 min. longer, during which time the temp. should gradually rise to about 69.5°. Plunge the flask at once into $H_2O$ at 20°. When the contents have cooled to about 35°, remove the thermometer from the flask, rinse it, and fill almost to the mark. Leave the flask in the bath at 20° for at least 30 min. longer and finally make up exactly to volume. Mix well and polarize the soln in a 200 mm tube provided with a lateral branch and a water jacket, maintaining a temp. of 20°. This reading must also be multiplied by 2 to obtain the invert reading. If it is necessary to work at a temp. other than 20°, which is permissible within narrow limits, the volumes must be completed and both direct and invert polarizations must be made at exactly the same temp.

Calculate sucrose by the following formula:

$$S=\frac{100\ (P-I)}{143+0.0676\ (m-13)-t/2}, \text{ in which}$$

$S$ = percentage of sucrose;
$P$ = direct reading, normal soln;
$I$ = invert reading, normal soln;
$t$ = temp. at which readings are made; and
$m$ = g of total solids in 100 cc of the invert soln read in the polariscope.

Determine the total solids as percentage by weight, as directed under **7**, and multiply this figure by the density at 20° as obtained from **XLII, 5.**

(**c**) *Inversion at room temperature*.—The inversion may also be accomplished as follows: (1) To 50 cc of the clarified soln, freed from Pb, add 10 cc of HCl (sp. gr. 1.1029 at 20/4° or 24.85° Brix at 20°) and set aside for 24 hours at a temp. not below 20°; or, (2) if the temp. is above 25°, set aside for 10 hours. Make up to 100 cc at 20° and polarize as directed under (**b**). Under these conditions the formula must be changed to the following:

$$S=\frac{100\ (P-I)}{143.2+0.0676\ (m-13)-t/2}.$$

## SUCROSE AND RAFFINOSE[18]

### *I. By Polarization Before and After Treatment with Two Enzyme Preparations—Official, first action*

**24** REAGENTS

(**a**) *Invertase soln* (*top yeast extract*).—Prepare as directed in **21.** This soln should be free from the enzyme melibiase. Its invertase activity should be at least as great as when used for the determination of sucrose in the absence of raffinose [**21**, (4)].

(**b**) *Invertase-melibiase soln* (*bottom yeast extract*).—Prepare as directed in **21**, using bottom fermenting yeast (brewers yeast) instead of bakers yeast. The invertase activity should be at least as great as in (**a**).

Test the melibiase activity of the soln as follows: Add 2 cc of the soln to be tested to 20 cc of a weakly acid melibiose soln polarizing +20.0°V and allow to stand 30 min. at about 20°. Then add sufficient $Na_2CO_3$ to render the soln slightly alkaline to litmus paper. A preparation suitable for the overnight hydrolysis of solns containing not more than 0.2 g of raffinose in 100 cc should have hydrolyzed 35% of the melibiose present under the conditions mentioned; a preparation suitable for the overnight hydrolysis of solns containing not more than 0.65 g of raffinose in 100 cc should have produced 50% hydrolysis of melibiose; and a preparation suitable for the overnight hydrolysis of solns containing 0.65–1.3 g of raffinose in 100 cc should have hydrolyzed at least 70% of the melibiose present under the above condition. The polarizations that correspond to 35, 50 and 70% hydrolysis of a melibiose soln polarizing before hydrolysis, +20°V are: +16.4°, +14.9° and +12.9°V, respectively.

### 25 DETERMINATION

In the analysis of sugar beet products, weigh the quantity of material specified in the following table, transfer to a 300 cc volumetric flask, add the quantity of basic Pb-acetate soln indicated in the table, and dilute to volume at 20°. Mix thoroly and filter thru fluted paper in a closely covered funnel, rejecting the first 25 cc of filtrate. When sufficient filtrate has collected, remove the Pb from the soln by adding $NH_4H_2PO_4$ in as small excess as possible (see table). This condition is readily determined after a little practice by the appearance of the $Pb_3(PO_4)_2$ precipitate, which usually flocculates and settles rapidly in the presence of a slight excess of the salt. Mix well and filter, again rejecting at least the first 25 cc of the filtrate. Make a direct polarization in a 200 mm tube at 20° unless the soln contains an appreciable quantity of invert sugar, in which case pipet a 50 cc portion of the Pb-free filtrate into a 100 cc flask, dilute with $H_2O$ to the mark, mix well, and polarize at 20°, preferably in a 400 mm tube. This reading, calculated to the normal weight of 26 g in 100 cc, and 200 mm tube length is the direct reading (*P*) of the formula given below for polarization before inversion.

*Quantities of sample and reagents required for clarification and deleading of beet sugar-house products*

| MATERIAL | QUANTITY PER 100 CC | BASIC LEAD ACETATE (55° BRIX) | AMMONIUM DIHYDROGEN PHOSPHATE |
|---|---|---|---|
| | *grams* | *cc* | *gram* |
| Cossettes[a] | 13 | 3 | 0.2 |
| Pulp | 100 cc[b] | 2–4 | 0.2 |
| Lime cake or sewer[c] | 26.5 | 1.5 | ....[d] |
| Thin juice | 52 | 2 | 0.2–0.3 |
| Thick juice | 26 | 4 | 0.3–0.4 |
| White massecuite | 13 or 26 | 3 or 6 | 0.3–0.7 |
| High wash sirup | 13 or 26 | 3 or 6 | 0.3–0.7 |
| High green sirup | 13 or 26 | 5 or 10 | 0.3–0.7 |
| Raw or remelt massecuite | 13 | 6 | 0.3–0.4 |
| Raw or remelt sugar | 26 | 3–4 | 0.3–0.4 |
| Sugar melter | 26 | 2–3 | 0.3–0.4 |
| Low wash sirup | 13 | 8–10 | 0.4–0.5 |
| Low green sirup or molasses | 13 | 10 | 0.4–0.5 |
| Saccharate cakes and milk (carbonated) | 26 | 4–6 | 0.3–0.4 |
| Steffen waste and wash waters | 78 or 50cc. | 2–3 | 0.2 |

[a] Usual method of extraction, 26 g in 201.2 cc.

[b] Dilute to 110 cc.

[c] Neutralize with acetic acid before adding basic Pb-acetate.

[d] Lime in soln will be partly precipitated by the phosphate, and it is necessary to add sufficient phosphate to complete the precipitation of both the lead and lime salts; hence no definite quantity can be specified.

Transfer two 50 cc portions of the Pb-free filtrate to 100 cc flasks. To one add 5 cc of invertase soln (top yeast extract) and to the other 5 cc of invertase-melibiase soln (bottom yeast extract), let stand overnight at atmospheric temp. (preferably not below 20°), dilute to volume, mix well, and polarize at 20°, preferably in a 400 mm jacketed tube. If a rapid hydrolysis is desired, add 10 cc of each of the enzyme solns to the 50 cc portions of deleaded filtrate in 100 cc flasks and place in a water bath at 50–55° for 40 min. Then add $Na_2CO_3$ until the soln is slightly alkaline to litmus paper, dilute to volume at 20°, mix well, and polarize at 20°, preferably in a 400 mm tube. Correct the invert readings for the optical activity of the enzyme soln and calculate the polarization to that of a normal weight soln of 26 g in 100 cc; also calculate the reading to a 200 mm tube length, if necessary.

Calculate the percentages of anhydrous raffinose and sucrose from the following formulas:

$$R = 1.354\ (A - B);$$

$$S = \frac{(P - 2.202A + 1.202B)\ 100}{132.12 - 0.00718\ \left[132.12 - (P - 2.202A + 1.202B)\right]},$$

Where $R$ = percentage of raffinose;
$S$ = percentage of sucrose;
$A$ = corrected polarization after top yeast hydrolysis; and
$B$ = corrected polarization after bottom yeast hydrolysis.

The quantities $A$ and $B$ are treated algebraically.

### 26 *II. By Polarization Before and After Inversion with HCl—Official*

(Of value chiefly in the analysis of beet products)

If the direct reading is more than 1° higher than the percentage of sucrose as calculated by the formula given under **23** (b), raffinose is probably present. Calculate sucrose and raffinose by the following formulas.[19]

When the polarizations are made at 20°—

$$S = \frac{0.514P - I}{0.844} \text{ and } R = \frac{0.33P + I}{1.563}, \text{ in which}$$

$P$ = direct reading, normal soln;
$I$ = invert reading, normal soln;
$S$ = percentage of sucrose; and
$R$ = percentage of anhydrous raffinose.

The following formulas[19] are applicable at all temps. other than 20°—

$$S = \frac{P\ (0.478 + 0.0018t_2) - I\ (1.006 - 0.0003t_1)}{(0.908 - 0.0032t_2)\ (1.006 - 0.0003t_1)} \text{ and}$$

$$R = \frac{P\ (0.43 - 0.005t_2) + I\ (1.006 - 0.0003t_1)}{(1.681 - 0.0059t_2)\ (1.006 - 0.0003t_1)}, \text{ in which}$$

$P$ = direct reading, normal soln;
$I$ = invert reading, normal soln;
$S$ = percentage of sucrose;
$R$ = percentage of anhydrous raffinose;
$t_1$ = temp. of the direct polarization; and
$t_2$ = temp. of the invert polarization.

### 27 SUCROSE BY DOUBLE DILUTION METHOD[20]—OFFICIAL

(Substances in which the volume of the combined insoluble matter and precipitate from clarifying agents is more than 1 cc from 26 g.)

Weigh a half-normal weight of the sample and dilute the soln to 100 cc, using the appropriate clarifier (basic Pb-acetate for dark colored confectionery or molasses and alumina cream for light colored confectionery). Also weigh a normal weight of the sample and dilute a second soln with the clarifier to 100 cc. Filter, and obtain direct polariscopic readings of both solns. Invert each soln as directed under **22**(**b**) or (**c**) or **23** (**b**) or (**c**) and obtain the respective invert readings.

The true direct polarization of the sample is equal to 4 times the direct polarization of the diluted soln less the direct polarization of the undiluted soln.

The true invert polarization is equal to 4 times the invert polarization of the diluted soln less the invert polarization of the undiluted soln.

Calculate the sucrose from the true polarizations thus obtained, using the formula under **22** or **23** corresponding to the method of inversion employed.

## CHEMICAL METHODS

### 28 FROM REDUCING SUGARS BEFORE AND AFTER INVERSION—OFFICIAL

Determine the reducing sugars (clarification having been effected with neutral Pb-acetate, never with basic Pb-acetate) as directed under **38** and calculate to invert sugar from **XLII, 9.** Invert the soln as directed under **22** (**b**) or (**c**), or **23** (**b**) or (**c**); exactly neutralize the acid; and again determine the reducing sugars, but calculate them to invert sugar from the table referred to above, using the invert sugar column alone. Deduct the percentage of invert sugar obtained before inversion from that obtained after inversion and multiply the difference by 0.95 to obtain the percentage of sucrose. The solns should be diluted in both determinations so that not more than 240 mg of invert sugar is present in the quantity taken for reduction It is important that all lead be removed from the soln with anhydrous powdered K-oxalate or $Na_2CO_3$ before reduction.

## Commercial Glucose[21] (Approximate)

## POLARIMETRIC METHODS

### 29 *Method I—Official*

(Substances containing little or no invert sugar)

Commercial glucose cannot be determined accurately owing to the varying quantities of dextrin, maltose, and dextrose present in the product. However, in sirups in which the quantity of invert sugar is so small as not to affect appreciably the result, commercial glucose may be estimated approximately by the following formula:

$$G = \frac{(a-S)\ 100}{211}, \text{ in which}$$

$G$ = percentage of commercial glucose solids;
$a$ = direct polarization, normal soln; and
$S$ = percentage of cane sugar.

Express the results in terms of commercial glucose solids polarizing +211°V. (This result may be recalculated in terms of commercial glucose of any Baumé reading desired.)

### 30 *Method II—Official*

(Substances containing invert sugar)

Prepare an inverted half-normal soln of the substance as directed under **23** (**b**), except to cool the soln after inversion, make neutral to phenolphthalein with NaOH soln, slightly acidify with HCl (1+5), and treat with 5–10 cc of alumina cream before making up to the mark. Filter, and polarize at 87° in a 200 mm jacketed metal tube, preferably silver. Multiply the reading by 200 and divide by the factor 196 to obtain the quantity of commercial glucose solids polarizing +211°V. (This result may be recalculated in terms of commercial glucose of any Baumé reading desired.)

## Invert Sugar

### CHEMICAL METHODS

### *I. Approximate Volumetric Method for Rapid Work—Tentative*

**31** REAGENTS

*Soxhlet's modification of Fehling's soln.*—Prepared by mixing immediately before use, equal volumes of (**a**) and (**b**).

(**a**) *Copper sulfate soln.*—Dissolve 34.639 g of $CuSO_4.5H_2O$ in $H_2O$, dilute to 500 cc, and filter thru prepared asbestos.

(**b**) *Alkaline tartrate soln.*—Dissolve 173 g of Rochelle salts and 50 g of NaOH in $H_2O$, dilute to 500 cc, allow to stand for 2 days, and filter thru prepared asbestos.

**32** STANDARDIZATION OF COPPER SOLUTION

Since the factor of calculation varies with the minute details of manipulation, every operator must determine a factor for himself, using a known soln of the pure sugar that he desires to determine and keeping the conditions the same as those used for the determination.

Standardize the soln for invert sugar in the following manner:

Dissolve 4.75 g of pure sucrose in 75 cc of $H_2O$, add 10 cc of HCl (sp. gr. 1.1029 at 20/4°), and invert as directed under **23** (**b**) or (**c**). Neutralize the acid with NaOH soln and dilute to 1 liter. 10 cc of this soln contains 0.050 g of invert sugar, which should reduce 10 cc of the reagent. The strength of the Cu soln should never be taken as a constant, but should be checked against the sugar.

**33** DETERMINATION

Place 10 cc of the reagent in a large test tube and add 10 cc of $H_2O$. Heat to boiling and add gradually small portions of the soln of the material to be tested until the Cu has been completely reduced, boiling after each addition to complete the reaction. It is necessary to boil 2 min. for complete reduction when the full quantity of sugar soln has been added in 1 portion. When the end is nearly reached and the quantity of sugar soln to be added can no longer be judged by the color of the soln, remove a small portion of the liquid and filter rapidly into a small porcelain crucible or on a test plate; acidify with dilute acetic acid; and test for Cu with dilute $K_4Fe(CN)_6$ soln. The sugar soln should be of such strength as to give a buret reading of 15–20 cc, and the number of successive additions should be as small as possible.

### *II. Lane-Eynon General Volumetric Method[22]—Tentative*

REAGENTS

The reagents and solns are described in **31**.

**34** STANDARDIZATION AND METHOD OF TITRATION

Pipet accurately 10 or 25 cc of mixed Soxhlet's reagent or pipet 5 or 12.5 cc of each of Soxhlet's solns (**a**) and (**b**) into a flask of 300–400 cc capacity. The quantity of Cu taken will differ slightly between the 2 methods of pipetting, and the method employed must be carried out consistently during standardization and determination. Prepare a standard soln of the pure sugar of such concentration that more than 15 cc and less than 50 cc will be required to reduce all the Cu. The titer may be calculated as follows: $\frac{\text{factor}}{\text{mg sugar in 1 cc}}$. Add almost the whole of the sugar soln required to effect reduction of all the Cu, so that not more than 0.5–1 cc is required later to complete the titration. Heat the cold mixture to boiling on a wire gauze and maintain in moderate ebullition for 2 min., lowering the flame sufficiently to avoid bumping. Without removing the flame add 2-5 drops of 1% aqueous methylene blue soln and complete the titration within a total boiling time of about 3 min. by small additions of sugar soln to decolorization of the indicator.

Multiply the titer by the number of mg in 1 cc of the standard soln to obtain the factor. Compare with the tabulated factor to determine the correction, if any, to be applied to the table. Small deviations from the tabulated factors may arise from variations in individual procedure or composition of reagents. If only approximate results (within 1%) are required the standardization may be omitted, provided the specifications of the analysis are rigidly observed.

### 35 DETERMINATION

If the approximate concentration of the sugar in the sample is unknown, proceed by the incremental method of titration. Add to 10 or 25 cc of Soxhlet's soln 15 cc of the sugar soln and heat to boiling over a wire gauze. Boil about 15 seconds and add rapidly further quantities of the sugar soln until only the faintest perceptible blue color remains. Then add 2–5 drops of methylene blue and complete the titration by adding the sugar soln dropwise. (The error resulting from this titration will not generally exceed 1 per cent.)

For analysis of higher precision repeat the titration, adding almost the whole of the sugar soln required to reduce all the Cu and proceed as described under **35**. In the table find the factor corresponding to the titer and apply the correction previously determined. Extimate as follows:

$$\frac{\text{factor} \times 100}{\text{titer}} = \text{mg of sugar in 100 cc.}$$

## *III. Scales Method*[23]

(Suitable when very small quantities of sugar are present)

### 36 REAGENTS

(a) *Benedict's solns.*—Dissolve 16 g of $CuSO_4.5H_2O$ in 125–150 cc of $H_2O$. Then dissolve 150 g of Na-citrate, 130 g of $Na_2CO_3$ (anhydrous), and 10 g of $NaHCO_3$ in about 650 cc of $H_2O$, heating to accelerate soln. Combine the 2 solns with stirring. Cool, make to 1 liter, and filter.

(b) *Iodine soln.*—0.04 *N.*

(c) *Thiosulfate soln.*—0.04 *N.*

(d) *Hydrochloric acid soln.*—60 cc of strong acid per liter.

(e) *Acetic acid soln.*—24 cc of glacial acid per liter.

(f) *Soluble starch soln.*

### PROCEDURE

Transfer 20 cc of Cu reagent (a) to a 300 cc Erlenmeyer flask fitted with a 2-holed rubber stopper. Add 10 cc of sugar soln containing less than 20 mg of reducing sugar. Place over a flame, bring to boiling in 4 min., and continue the boiling for exactly 3 min. (Approximate conditions, flame 50 mm, cone 20 mm, asbestos gauze 30 mm above burner. If preferred, an electric hot plate may be used, in which case a period of 5 min. is required to raise the soln to the boiling point.) At the expiration of 3 min. from the beginning of the boiling, cool rapidly by holding under a cold water faucet, add 100 cc of the dilute acetic acid from a graduate, and transfer an exactly measured 0.04 $N$ I soln (b). Add 25 cc of HCl (d) from a pipet held against the side of the flask, and agitate to distribute the acid rapidly. Rotate the flask for 1 min. to insure the soln of all $Cu_2Cl_2$. Titrate excess I with thiosulfate soln.

For amounts less than 20 mg of sugar each cc of thiosulfate will represent a constant quantity of sugar; for dextrose, approximately 1.12 mg per cc. (For accurate work the analyst should determine the conversion factor for the particular conditions under which he is working, by using control solns of the pure sugars under examination.)

## *IV. Munson and Walker General Method*[24]*—Official*

**37** REAGENTS

(a) *Asbestos.*—Digest the asbestos, which should be the amphibole variety, with HCl (1+3) for 2-3 days. Wash free from acid, digest for a similar period with 10% NaOH soln, and then treat for a few hours with hot alkaline tartrate soln (old alkaline tartrate solns that have stood for some time may be used for this purpose) of the strength used in sugar determinations. Wash the asbestos free from alkali; digest for several hours with $HNO_3$ (1+3); and, after washing free from acid, shake with $H_2O$ into a fine pulp. In preparing the Gooch crucible, make a film of asbestos $\frac{1}{4}$ inch thick and wash thoroly with $H_2O$ to remove fine particles of asbestos. If the precipitated $Cu_2O$ is to be weighed as such, wash the crucible with 10 cc of alcohol, then with 10 cc of ether; dry for 30 min. at 100°; cool in a desiccator; and weigh.

(b) The other reagents and solns used are described under **31.**

**38** PRECIPITATION OF CUPROUS OXIDE

Transfer 25 cc of each of the $CuSO_4$ and alkaline tartrate solns to a 400 cc beaker of alkali-resistant glass and add 50 cc of the reducing sugar soln, or if a smaller volume of sugar soln is used, add $H_2O$ to make the final volume 100 cc. Heat the beaker on an asbestos gauze over a Bunsen burner, regulate the flame so that boiling begins in 4 min., and continue the boiling for exactly 2 min. (It is important that these directions be strictly observed. To regulate the burner for this purpose it is advisable to make preliminary tests, using 50 cc of the reagent and 50 cc of $H_2O$ before proceeding with the actual determination.) Keep the beaker covered with a watch-glass during the heating. Filter the hot soln at once thru an asbestos mat in a porcelain Gooch crucible, using suction. Wash the precipitate of $Cu_2O$ thoroly with $H_2O$ at a temp. of about 60° and either weigh directly as $Cu_2O$ as directed under **39**, or determine the quantity of reduced Cu by one of the methods described under **41**-**44**, respectively. Conduct a blank determination, using 50 cc of the reagent and 50 cc of $H_2O$, and if the weight of $Cu_2O$ obtained exceeds 0.5 mg, correct the result of the reducing sugar determination accordingly. The alkaline tartrate soln deteriorates on standing, and the quantity of $Cu_2O$ obtained in the blank increases.

### DETERMINATION OF REDUCED COPPER

### 39 *Direct Weighing of Cuprous Oxide*

(This method should be used only for determinations in solns of reducing sugars of comparatively high purity. In products containing large quantities of mineral or organic impurities (including sucrose), determine the Cu of the $Cu_2O$ by one of the methods described under **41–44**, since the $Cu_2O$ is very likely to be contaminated with foreign matter.)

Prepare a Gooch crucible as directed under **37** (**a**). Collect the precipitated $Cu_2O$ on the mat as directed under **38** and wash thoroly with hot $H_2O$, then with 10 cc of alcohol, and finally with 10 cc of ether. Dry the precipitate for 30 min. in a water oven at the temp. of boiling $H_2O$, cool, and weigh. Calculate the weight of metallic Cu, using the factor 0.8882. Obtain from **XLII, 9,** the weight of invert sugar equivalent to the weight of Cu.

The number of mg of Cu reduced by a given quantity of reducing sugar varies, depending upon whether or not sucrose is present. In the tables the absence of sucrose is assumed except in the entries under invert sugar, where, in addition to the column for invert sugar alone, there are given one column for mixtures of invert sugar and sucrose containing 0.4 g of total sugar in 50 cc of soln and one column for invert sugar and sucrose when the 50 cc of soln contains 2 g of total sugar. Two entries are also given under lactose and sucrose mixtures, showing proportions of 1 part lactose to 4 and 12 parts of sucrose, respectively.

### *Volumetric Thiosulfate Method*[25]

### 40 REAGENTS

*Standard thiosulfate soln.*—Prepare a soln of $Na_2S_2O_3$ containing 19 g of pure crystals in 1 liter. Weigh accurately about 0.2 g of pure Cu and place in a flask of 250 cc capacity. Dissolve by warming with 5 cc of a mixture of equal volumes of strong $HNO_3$ and $H_2O$. Dilute to 50 cc, boil to expel the red fumes, add a slight excess of strong Br water, and boil until the Br is completely driven off. Cool, and add a strong NaOH soln with agitation until a faint turbidity of $Cu(OH)_2$ appears (about 7 cc of a 25% NaOH soln is required). Discharge the turbidity with a few drops of 80% acetic acid and add 2 drops in excess. (The soln should now occupy a volume of 50–70 cc.) Add 10 cc of 30% KI soln. Titrate at once with the thiosulfate soln until the brown tinge becomes weak and add sufficient starch indicator [**VI, 3** (**e**)] to produce a marked blue coloration. Continue the titration cautiously until the color changes toward the end to a faint lilac. (If at this point the thiosulfate is added dropwise and a little time is allowed for complete reaction after each addition, no difficulty is experienced in determining the end point within a single drop.) 1 cc of the thiosulfate soln = about 0.005 g of Cu.

### 41 DETERMINATION

After washing the precipitated $Cu_2O$, cover the Gooch with a watch-glass and dissolve the oxide by means of 5 cc of warm $HNO_3$ (1+1) poured under the watch-glass with a pipet. Collect the filtrate in a 250 cc flask and wash the watch-glass and the Gooch free from Cu, using about 50 cc of $H_2O$. Boil to expel red fumes; add a slight excess of Br water; boil off the Br completely; and proceed as directed under **40**, beginning with "Cool and add a strong NaOH soln. . . ."

### 42 *Volumetric Permanganate Method*

Filter and wash the $Cu_2O$ as directed under **38**. Transfer the asbestos film to the beaker, add about 30 cc of hot $H_2O$, and beat the precipitate and asbestos thoroly. Rinse the crucible with 50 cc of a hot saturated soln of $Fe_2(SO_4)_3$ in 20% $H_2SO_4$, receiving the rinsings in the beaker containing the precipitate. After the $Cu_2O$ is dissolved, wash the soln into a large Erlenmeyer flask and immediately titrate with a standard soln of $KMnO_4$, 1 cc of which should be equivalent to 0.010 g of Cu. Standardize this soln by making 6 or more determinations with the same sugar soln, titrating ½ of the precipitates obtained and determining the Cu in the others by electrolysis. The average weight of Cu obtained by electrolysis, divided by the average number of cc of $KMnO_4$ soln required for the titrations, gives the weight of Cu equivalent to 1 cc of the standard $KMnO_4$ soln. A soln standardized with iron or oxalic acid will give too low a result, but agreement with electrolytic determination will be obtained if pure Na-oxalate is used and is made acid with $H_2SO_4$.

### 43 *Electrolytic Deposition from Sulfuric and Nitric Acid Solution*[26]

Decant the hot soln thru an asbestos mat in a Gooch crucible and wash the beaker and precipitate thoroly with hot $H_2O$. Transfer the asbestos film from the crucible to the beaker by means of a glass rod, and rinse the crucible with about 30 cc of a boiling mixture containing 65 cc of $H_2SO_4$ and 50 cc of $HNO_3$ per liter. Heat, and agitate until soln is complete and the oxides of N have been volatilized. Filter, and transfer to a weighed Pt dish. Dilute to about 100 cc and deposit the Cu by electrolysis at 20–30°, using about 2.5 volts and a current density of about 0.5 ampere. Cover the dish with a split watch-glass to avoid loss by spattering. Electrolysis requires about 14 hours, but it may be allowed to continue overnight. Test 1 cc with strong $H_2S$ soln for complete deposition. Wash thoroly with $H_2O$; then break the current, wash with alcohol and ether successively, dry at about 50°, and weigh.

If preferred, the electrolysis can be conducted in a beaker, the Cu being deposited upon a weighed Pt foil or Pt gauze.

### 44 *Reduction in Hydrogen*

(This method should be used only for determinations in solns of reducing sugars of comparatively high purity.)

Deposit an asbestos film on a perforated Pt disk or cone contained in a hard glass filtering tube, wash free from loose fibers, dry, and weigh. Thru this tube, previously moistened, filter the $Cu_2O$ immediately, using suction. Transfer the $Cu_2O$ to the tube thru a removable funnel and wash thru with hot $H_2O$, alcohol, and ether successively. After drying connect the tube with a supply of dry H, heat gently until the $Cu_2O$ is completely reduced to metallic Cu, cool in the current of H, and weigh. If preferred, a Gooch crucible may be used for the filtration.

## *Herzfeld Gravimetric Methods—Official*

### *Method I*

(For materials containing 1.5% or less of invert sugar and 98.5% or more of sucrose.)

### 45 REAGENTS

The reagents and solns used are described under **31** and **37**.

### 46 DETERMINATION

Prepare the soln of the material to be examined to contain 20 g in 100 cc, clarify with neutral Pb-acetate, and remove the excess of Pb with $Na_2CO_3$. Place 50 cc of the reagent and 50 cc of the sugar soln in a 250 cc beaker. Heat this mixture at such a rate that approximately 4 min. is required to bring it to the boiling point and boil for exactly 2 min. Add 100 cc of cold recently boiled $H_2O$. Filter immediately thru asbestos and determine the Cu by one of the methods described under **41–44,** respectively. Obtain the corresponding percentage of invert sugar from **XLII, 10.**

### *Method II*

(For materials containing more than 1.5% of invert sugar and less than 98.5% of sucrose.)

### 47 REAGENTS

The reagents and solns used are described under **31** and **37.**

### 48 DETERMINATION

Prepare a soln of suitable concentration of the material to be examined, clarify with neutral Pb-acetate, and remove the excess of Pb. Prepare a series of solns in large test tubes by adding 1, 2, 3, 4, and 5 cc of this soln to each tube successively. Add 5 cc of the reagent to each, heat to boiling, boil 2 min., and filter. Note the volume of sugar soln that gives the filtrate lightest in tint, but still distinctly blue. Place 20 times this volume of the sugar soln in a 100 cc flask, dilute to the mark, and mix well. Use 50 cc of the soln for the determination, which is conducted as described under **46.** For the calculation of the result use the following formulas and table of factors of Meissl and Hiller, **XLII, 11.**,

Let $Cu$ = the weight of Cu obtained;
$P$ = the polarization of the sample;
$W$ = the weight of the sample in the 50 cc of the soln used for the determination; and
$F$ = the factor obtained from the table for the conversion of Cu to invert sugar.

Then $\frac{Cu}{2} = Z$, approximate weight of invert sugar;

$Z \times \frac{100}{W} = Y$, approximate percentage of invert sugar;

$\frac{100\,P}{P+Y} = R$, approximate percentage of sucrose in mixture of sugars;

$100 - R = I$, approximate percentage of invert sugar; and

$\frac{CuF}{W}$ = percentage of invert sugar.

The factor $F$ for calculating Cu to invert sugar is then found from **XLII, 11.** *Example:* The polarization of a sugar is 86.4, and 50 cc of soln containing 3.256 g of sample gives 0.290 g of Cu.

$$\frac{Cu}{2} = \frac{0.290}{2} = 0.145 = Z.$$

$$\frac{Z \times 100}{W} = 0.145 \times \frac{100}{3.256} = 4.45 = Y.$$

$$\frac{100\,P}{P+Y} = \frac{8460}{86.4+4.45} = 95.1 = R.$$

$$100 - R = 100 - 95.1 = I = 4.9.$$
$$R{:}I = 95.1{:}4.9.$$

By consulting the table it will be seen that the vertical column headed 150 is nearest to $Z$, 145, and the horizontal column headed 95: 5 is nearest to the ratio of $R$ to $I$, 95.1:4.9. Where these columns meet, there is found the factor 51.2, which enters into the final calculation:

$$\frac{CuF}{W} = \frac{0.290 \times 51.2}{3.256} = 4.56\% \text{ of invert sugar.}$$

## Maltose

### CHEMICAL METHODS

**49** *General Volumetric Method—Tentative*

Proceed as directed under **35**, referring the titer to **XLII, 15** or **16.**

**50** *General Gravimetric Method—Official*

Proceed as directed under **38** and obtain from **XLII, 9** the weight of maltose equivalent to the weight of Cu reduced.

### *Wein Method—Official*

**51** REAGENTS

The reagents and solns used are described under **31** and **37.**

**52** DETERMINATION

Place 50 cc of the reagent in a beaker and heat to the boiling point. When boiling briskly, add 25 cc of the maltose soln containing not more than 0.250 g of maltose and boil for 4 min. Filter immediately thru asbestos and determine, by one of the methods given under **39, 41–44,** respectively, the quantity of Cu reduced. Obtain from **XLII, 12,** the weight of maltose equivalent to the weight of Cu.

## Lactose

### CHEMICAL METHODS

**53** *General Volumetric Method—Tentative*

Proceed as directed under **35,** referring the titer to **XLII, 15** or **16.**

**54** *General Gravimetric Method—Official*

Proceed as directed under **38** and obtain from **XLII, 9,** the weight of lactose equivalent to the weight of Cu reduced.

### *Soxhlet-Wein Method—Official*

**55** REAGENTS

The reagents and solns used are described under **31** and **37.**

**56** DETERMINATION

Place 50 cc of the reagent in a beaker and heat to the boiling point. When boiling briskly, add 100 cc of the lactose soln containing not more than 0.300 g of lactose and boil for 6 min. Filter immediately thru asbestos and determine by one of the methods given under **39, 41–44,** respectively, the quantity of Cu reduced. Obtain from **XLII, 13,** the weight of lactose equivalent to the weight of Cu found.

## Dextrose

## CHEMICAL METHODS

### **57** *Approximate Volumetric Method for Rapid Work—Tentative*

Proceed as directed under **33.** Standardize the reagent against pure dextrose.

### **58** *General Volumetric Method—Tentative*

Proceed as directed under **35,** referring the titer to **XLII, 15** or **16.**

### **59** *General Gravimetric Method—Official*

Proceed as directed under **38** and obtain from **XLII, 9,** the weight of dextrose equivalent to the weight of Cu reduced.

### *Allihn Gravimetric Method—Official*

**60** REAGENTS

*Allihn's modification of Fehling's soln.*—Prepare by mixing, immediately before use, equal volumes of (**a**) and (**b**).

(**a**) *Copper sulfate soln.*—Dissolve 34.639 g of $CuSO_4.5H_2O$ in $H_2O$ and dilute to 500 cc.

(**b**) *Alkaline tartrate soln.*—Dissolve 173 g of Rochelle salts and 125 g of KOH in $H_2O$ and dilute to 500 cc.

**61** DETERMINATION

Place 30 cc of the $CuSO_4$ soln, 30 cc of the alkaline tartrate soln, and 60 cc of $H_2O$ in a beaker, and heat to boiling. Add 25 cc of the soln of the material to be examined, prepared so as to contain not more than 0.25 g of dextrose, and boil exactly 2 min., keeping the beaker covered. Filter immediately thru asbestos and obtain the weight of Cu by one of the methods given under **39, 41–44,** respectively. Obtain the corresponding weight of dextrose from **XLII, 14.**

## 62 Reducing Sugars Other Than Dextrose—Official

Proceed as directed under **61** and multiply the weight of dextrose found by the following factors:

Levulose, 1.093; Invert sugar, 1.044; Arabinose, 0.969; Xylose, 1.017; and Galactose, 1.114.

## CONFECTIONERY

### 63 PREPARATION OF SAMPLE—OFFICIAL

The preparation of the sample for analysis depends upon the character of the product and the determinations to be made. When the composition of the entire sample is desired, grind and mix thoroly. When the sample is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with a knife or other mechanical means as completely as possible and grind and mix each portion thoroly.

### 64 MOISTURE—OFFICIAL

Proceed as directed under **2.**

### 65 ASH—OFFICIAL

Proceed as directed under **8** or **9.**

### 66 MINERAL CONSTITUENTS

Proceed as directed under **XII.**

### 67 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under **12.**

### 68 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as directed under **13.**

### 69 ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Proceed as directed under **14.**

### 70 MINERAL ADULTERANTS IN THE ASH—TENTATIVE

Proceed as directed under **15.**

### 71 Nitrogen—Official

Determine N in 2–5 g of the material as directed under **II, 19, 22,** or **24,** using a larger quantity of $H_2SO_4$ if necessary for complete digestion.

## Sucrose

## POLARIMETRIC METHODS

### 72 SUCROSE IN THE ABSENCE OF RAFFINOSE

Proceed as directed under **22, 23,** or **27.**

## CHEMICAL METHODS

### 73 *By Reducing Sugars Before and After Inversion—Official*

Proceed as directed under **28.**

### 74 Commercial Glucose—Official

Proceed as directed under **28** or **29.**

### 75 Starch—Tentative

Measure 25 cc of a soln of uniform mixture (representing 5 g of the sample) into a 300 cc beaker, or introduce 5 g of the finely ground sample (previously extracted with ether if the sample contains much fat) into the beaker; add sufficient $H_2O$ to make the volume 100 cc; heat to about 60° (avoiding if possible gelatinizing the starch); and allow to stand for about an hour, stirring frequently to secure complete soln of the sugars. Transfer to a wide-mouthed bottle, rinse the beaker with a little warm $H_2O$, cool, add an equal volume of 95% alcohol, mix, and allow to stand at least an hour. Centrifugalize until the precipitate is closely packed on the bottom of the bottle and decant the supernatant liquid thru a hardened filter. Wash the precipitate with successive 50 cc portions of alcohol, 50% by volume, by centrifugalizing and decanting thru the filter until 3 or 4 drops of the washings give no test for sugar with alpha-naphthol as described under **124**. Transfer the residue from the bottle and the hardened filter to a large flask and determine starch as directed under **XXVII, 23.**

## ETHER EXTRACT

### 76 *I. Continuous Extraction Method—Tentative*

(1) Measure 25 cc of a 20% mixture or soln into a very thin, readily frangible glass evaporating shell, or a thin lead or tin foil dish containing 5–7 g of freshly ignited asbestos fiber; or (2) if possible to obtain a uniform sample, weigh 5 g of the mixed finely divided sample into a dish and wash with $H_2O$ upon the asbestos in the evaporating shell, using, if necessary, a small portion of the asbestos fiber on a stirring rod to transfer the last traces of the sample from the dish to the shell. Dry to constant weight at 100°, cool, wrap the glass dish loosely in smooth paper, crush into rather small fragments between the fingers, and transfer carefully the crushed mass, including the paper, to an extraction tube or a fat extraction cartridge. If a metal dish is used, cut it into small pieces and place in the extraction tube. Extract with anhydrous ether or petroleum ether (b. p. 45–60 and without weighable residue) in a continuous extraction apparatus for at least 25 hours. In most cases it is advisable to remove the substance from the extractor after the first 12 hours, grind with sand to a fine powder, and re-extract for the remaining 13 hours. Transfer the extract to a weighed flask, evaporate the solvent, and dry to constant weight at 100°.

### 77 *II. Roese-Gottlieb Method—Tentative*

Introduce 4 g of the material, or a quantity of a uniform soln equivalent to this weight of the dry substance, into a Röhrig tube or a similar apparatus; make up to a volume of 10 cc with $H_2O$; add 1.25 cc of $NH_4OH$; and mix thoroly. Add 10 cc of 95% alcohol and mix; then add 25 cc of ether and shake vigorously for half a min.; and finally add 25 cc of petroleum ether (b. p. below 60°) and shake again for half a min. Allow to stand for 20 min. or until the separation of the liquids is complete. Draw off as much as possible of the ether-fat soln (usually 0.5–0.8 cc will be left) into a weighed flask thru a small, rapid filter. The flask should be weighed with a similar one as a counterpoise. Again extract the liquid remaining in the tube, this time with 15 cc each of ether and petroleum ether; shake vigorously half a min. with each solvent; and allow to settle. Proceed as above, washing the tip of the spigot and the filter with a few cc of a mixture of equal parts of the two ethers (previously mixed and freed from deposited $H_2O$). For a greater degree of accuracy the extraction must be repeated. If the previous ether-fat solns have been drawn off closely, this third extraction usually yields not more than about 1 mg of fat, or about 0.02% on a 4 g charge. Evaporate the ether slowly on a steam bath and then dry the fat in a boiling water oven until the loss in weight ceases. Test the purity of the fat by dissolving in a little petroleum ether. Should a residue remain, wash the fat out completely with petroleum ether, dry the residue, weigh, and deduct the weight.

### 78 PARAFFIN—TENTATIVE

Add to the ether extract in the flask, as obtained under **76** or **77**, 10 cc of 95% alcohol and 2 cc of NaOH soln (1+1); connect the flask with a reflux condenser; and heat for an hour on a water bath, or until saponification is complete. Remove the condenser and allow the flask to remain on the bath until the alcohol is evaporated and the residue is dry. Dissolve the residue as completely as possible in about 40 cc of $H_2O$ and heat on the bath, shaking frequently. Wash into a separatory funnel, cool, and extract with 4 successive portions of petroleum ether, collecting the extracts in a weighed flask or capsule. Evaporate the petroleum ether and dry to constant weight at 100°. Any phytosterol or cholesterol present in the fat would be extracted with the paraffin, but the quantity is so insignificant that it may be disregarded generally.

### 79 ALCOHOL IN SIRUPS USED IN CONFECTIONERY ("BRANDY DROPS")—OFFICIAL

Collect in a beaker the sirup from a sufficient number of pieces to yield 30–50 g, strain into a weighed beaker, and weigh. Introduce the sirup into a 250–300 cc distillation flask; dilute with half its volume of $H_2O$; attach the flask to a vertical condenser; and distil almost 50 cc, or as much of the liquid as possible without causing charring. Foaming may be prevented by adding to the contents of the distillation flask a little tannin, or a piece of paraffin about the size of a pea. Cool the distillate, make up to volume with $H_2O$, mix well, and ascertain the specific gravity of the liquid by means of a pycnometer. Obtain the corresponding weight of alcohol in the 50 cc of distillate from **XLII, 19, 20** or **21.** Calculate by weight the percentage of alcohol in the candy filling.

### 80 COLORING MATTER—TENTATIVE

Proceed as directed under **XXI.**

### 81 METALS—TENTATIVE

Proceed as directed under **XXIX.**

## HONEY[27]

### 82 PREPARATION OF SAMPLE—OFFICIAL

(**a**) *Liquid or strained honey.*—If the sample is free from granulation, mix thoroly by stirring or shaking before weighing portions for the analytical determination. If the honey is granulated, place the container, having the stopper loose, in a water bath and heat at a temp. not exceeding 50° until the sugar crystals dissolve. Mix thoroly, cool, and weigh portions for the analytical determinations. If foreign matter, such as wax, sticks, bees, particles of comb, etc., is present, heat the sample to 40° in a water bath and strain thru cheese-cloth before weighing portions for analysis.

(**b**) *Comb honey.*—Cut across the top of the comb, if sealed, and separate completely from the comb by straining thru a 40-mesh sieve. When portions of the comb or wax pass thru the sieve, heat the sample as in (**a**) and strain thru cloth. If the honey is granulated in the comb, heat until the wax is liquefied; stir; cool; remove the wax; and take the clear liquid for analysis.

### 83 MOISTURE—OFFICIAL

Proceed as directed under **4**, using a weighed quantity of the sample sufficient to yield approximately 1 g of solids; adding, if necessary, a few cc of $H_2O$ to incorporate thoroly with the sand; and drying at 70° under a pressure of not to exceed 100 mm of Hg.

### 84 ASH—OFFICIAL

Weigh 5–10 g of honey into a Pt dish, add a few drops of pure olive oil to prevent spattering, heat carefully until swelling ceases, and ignite at a temp. not above dull redness until a white ash is obtained.

### 85 SOLUBLE ASH—OFFICIAL

Proceed as directed under **12**.

### 86 ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under **13**.

### 87 DIRECT POLARIZATION—TENTATIVE

(**a**) *Immediate direct polarization.*—Transfer 26 g of the honey to a 100 cc flask with $H_2O$, add 5 cc of alumina cream, dilute to the mark with $H_2O$ at 20°, filter, and polarize immediately in a 200 mm tube.

(**b**) *Constant direct polarization.*—Complete the mutarotation as directed under **20**. If necessary to conserve the sample, the soln from the tube used in the immediate direct polarization (**a**) may be returned to the flask. Make the final reading at 20° in a 200 mm tube.

(**c**) *Mutarotation.*—The difference between (**a**) and (**b**) is a measure of the mutarotation.

(**d**) *Direct polarization at 87°.*—Polarize the soln obtained under (**b**) at 87° in a jacketed 200 mm metal tube, preferably of silver.

### 88 INVERT POLARIZATION—TENTATIVE

(**a**) *At 20°.*—Invert 50 cc of the soln obtained under **87** as directed under **22** (**b**) or (**c**) or **23** (**b**) or (**c**), and polarize at 20° in a 200 mm tube.

(**b**) *At 87°.*—Polarize the soln obtained under (**a**) at 87° in a 200 mm metal tube, preferably of silver.

### 89 REDUCING SUGARS—OFFICIAL

Dilute 10 cc of the soln used for direct polarization, **87**, to 250 cc and determine reducing sugars in 25 cc of this soln by one of the methods given under **36, 38, 46, 48**, or **62**, respectively. Calculate the result to percentage of invert sugar.

### 90 SUCROSE—OFFICIAL

(**a**) Calculate from the data given in **87** (**b**) and **88** (**a**) if inversion is conducted as directed under **22** (**b**) or (**c**). Use the formula as given in **22** (**b**).

(**b**) Proceed as directed under **30**. Determine reducing sugars after inversion by diluting 10 cc of the soln obtained under **88** with a small quantity of $H_2O$, neutralizing with $Na_2CO_3$, and making up to 250 cc with $H_2O$. Use 50 cc of this soln, making the determination as directed in **89**.

### 91 LEVULOSE—TENTATIVE

Multiply the direct reading at 87°, **87** (**d**), by 1.0315 and subtract the product from the constant direct polarization at 20°, **87** (**b**); divide the difference by 2.3919 to obtain the grams of levulose in a normal weight of the honey. From this figure calculate the percentage of levulose in the original sample.

### 92 DEXTROSE—TENTATIVE

To obtain the approximate percentage of dextrose, subtract the percentage of levulose obtained under **91** from the percentage of invert sugar found under **89**.

The dextrose can be determined more accurately by multiplying the percentage of levulose, as found under **91**, by the factor 0.915, which gives its dextrose equivalent in Cu reducing power. Subtract the figure obtained from that of the reducing sugars, **89**, calculated as dextrose, to obtain the percentage of dextrose in the sample. (Owing to the difference in the reducing powers of different sugars, the sum of the dextrose thus found and the levulose as obtained under **91** will be greater than the quantity of invert sugar obtained under **89**.

### 93 DEXTRIN (APPROXIMATE)—TENTATIVE

Using not more than 4 cc of $H_2O$, transfer 8 g of the sample (4 g in the case of dark colored honey dew honey) to a 100 cc flask by allowing the sample to drain from the weighing dish into the flask and then dissolving the residue in 2 cc of $H_2O$. After adding this soln to the contents of the flask, rinse the weighing dish with two 1 cc portions of $H_2O$, adding a few cc of absolute alcohol each time before decanting. Fill the flask to the mark with absolute alcohol, shaking constantly. Set the flask aside until the dextrin has collected on the sides and bottom and the liquid is clear. Decant the clear liquid thru a filter paper and wash the residue in the flask with 10 cc of 95% alcohol, pouring the washings thru the same filter. Dissolve the dextrin in the flask with boiling $H_2O$ and filter thru the filter paper already used, receiving the filtrate in a weighed dish prepared as directed under **4**. Rinse the flask and wash the filter a number of times with small portions of hot $H_2O$, evaporate on a water bath, and dry to constant weight at 70° under a pressure of not to exceed 100 mm of Hg.

After determining the weight of the alcohol precipitate, dissolve the latter in $H_2O$ and make up to definite volume, using 50 cc of $H_2O$ for each 0.5 g of precipitate or part thereof.

Determine reducing sugars in the soln both before and after inversion as directed under **30**, expressing the results as invert sugar. Calculate sucrose from the results thus obtained and subtract the sum of the reducing sugars before inversion and sucrose from the weight of the total alcohol precipitate to obtain the weight of the dextrin.

### 94 FREE ACID—OFFICIAL

Dissolve 10 g of the honey in $H_2O$ and titrate with 0.1 $N$ NaOH soln, using phenolphthalein indicator. Express the results in terms of cc of 0.1 $N$ NaOH required to neutralize 100 g of the sample.

## COMMERCIAL GLUCOSE

### 95 *I. Qualitative Test—Tentative*

Dilute the honey with $H_2O$ in the proportion of 1 to 1 and add a few cc of I soln (1 g of I, 3 g of KI, 50 cc of $H_2O$). In the presence of commercial glucose the soln turns red or violet, the depth and character of the color depending upon the quality and nature of the glucose employed. A blank test with a pure honey of about the same color should be made in order to secure an accurate color comparison. Should the honey be dark and the percentage of glucose very small, precipitate the dextrin that may be present by adding several volumes of 95% alcohol. Allow to stand until the precipitate settles (do not filter), decant the liquid, dissolve the residue of dextrins in hot $H_2O$, cool, and apply the above test to this soln. A negative result is not proof of the absence of commercial glucose, as some glucose, especially of high conversion, does not give any reaction with I.[28]

96

### *II. Quantitative Method—Tentative*

An approximate determination can be made by Browne's formula as follows: Multiply the difference in the polarizations of the invert soln at 20° and 87° by 77 and divide this product by the percentage of invert sugar found in the sample after inversion. Multiply the quotient by 100 and divide the product by 26.7 to obtain the percentage of honey in the sample; 100% minus the percentage of honey gives the percentage of glucose.

## COMMERCIAL INVERT SUGAR[29]

### *I. Resorcin Test[30]—Tentative*

97

#### REAGENT

*Resorcin soln.*—Dissolve 1 g of resorcin in 100 cc of HCl.

98

#### DETERMINATION

Introduce 10 cc of a 50% honey soln into a test tube and add 5 cc of ether. Shake gently and allow to stand for some time until the ether layer is clear. Transfer 2 cc of this clear ether soln to a small test tube and add a large drop of the recently prepared resorcin soln. Shake, and note the color immediately. A cherry red color appearing at once indicates the presence of commercial invert sugar. Yellow to salmon shades of color have no significance.

### *II. Aniline Chloride Test[31]—Tentative*

(Not applicable to dark colored honeys)

99

#### REAGENT

*Aniline chloride soln.*—To 100 cc of C. P. aniline add 30 cc of 25% HCl.

100

#### DETERMINATION

Introduce 5 g of the honey into a porcelain dish and add 2.5 cc of the recently prepared aniline reagent. In the presence of commercial invert sugar, the reagent assumes immediately an orange-red color turning dark red. Yellow to salmon shades of color have no significance.

The resorcin test and the aniline chloride test,[32] when positive, may be considered conclusive evidence of the presence of commercial invert sugar in honey provided the honey has not been stored for some time after having been heated above 160°F.

The resorcin test and the aniline chlorine test, when negative, may not be regarded as conclusive evidence of the absence of commercial invert sugar sirup in honey.

102

## DIASTASE[33]—TENTATIVE

Mix 1 part of honey with 2 parts of sterile $H_2O$. Treat 10 cc of this soln with 1 cc of 1% soluble starch soln and digest at 45° for an hour. At the end of this time test the mixture with 1 cc of I soln (1 g of I, 2 g of KI, 300 cc of $H_2O$). Treat another 10 cc portion of the honey soln, mixed with 1 cc of the soluble starch soln without heating to 45°, with the reagent and compare the colors produced. If the original honey has not been heated sufficiently to destroy the diastase, an olive-green or brown coloration will be produced in the mixture that has been heated at 45°. Heated or artificial honey becomes blue.

## MAPLE PRODUCTS

### 103 PREPARATION OF SAMPLE*

#### (*a*) *Maple Sirup—Tentative*

1. *For moisture determination.*—If the sample contains no sugar crystals or suspended matter, decant sufficient of the clear sirup for use in the determination. If sugar crystals are present, redissolve them by heating. If suspended matter is present, filter the sample thru cotton wool.

2. *For other determinations.*—If sugar crystals are present, redissolve them by heating. If other sediment is present, distribute it evenly thru the sirup by shaking. Transfer approximately 100 cc of the sirup, with its suspended sediment, to a casserole or beaker, add $\frac{1}{4}$ the volume of $H_2O$, and evaporate over a flame. When the temp. of the boiling sirup approaches 104°, draw a small quantity into a thin-walled pipet of about 1 cc capacity, and cool this to room temp. in running $H_2O$. Wipe the outside of the pipet, allow the possibly diluted sirup in the point to escape, and make a refractometric measurement of the solids content of the cooled sirup. Repeat this procedure from time to time until a reading corresponding to 64.5% solids ($n_{20} = 1.4521$) is obtained. Filter the sirup thru a filter which will allow the 100 cc to pass within 5 min. and adjust the filtrate to $65.0 = 0.5\%$ solids (refractometric) by the thoro mixing with the appropriate quantity of $H_2O$. To facilitate the filtration a hot water funnel, suction or a "filter-aid" may be employed.

#### (*b*) *Maple Sugar and Other Solid or Semi-solid Products—Tentative*

1. *For moisture determination.*—Grind in a mortar, if necessary, and mix thoroly.

2. *For other determinations.*—Prepare a sirup by dissolving approximately 100 g of the sample in about 150 cc of hot $H_2O$, boil to a temp. of 104°, and filter thru cotton wool. Determine moisture in the prepared sirup as directed under **104** (a) and calculate results, other than for moisture, to a moisture-free basis.

### 104 MOISTURE—OFFICIAL

#### (*a*) *Maple Sirup*

Proceed as directed under **4** or **7**, preferably the latter, using the sample prepared as directed under **103** (*a*) (1).

#### (*b*) *Maple Sugar, Maple Cream, etc.*

Proceed as directed under **83**, using the sample prepared as directed under **103** (*b*) (1).

### 105 POLARIZATION—OFFICIAL

#### *Direct Polarization*

Proceed as directed under **22** (**a**) or **23** (**a**).

#### *Invert Polarization*

(**a**) *At 20°.*—Proceed as directed under **22** (**b**) or (**c**) or **23** (**b**) or (**c**).
(**b**) *At 87°.*—Proceed as directed under **30**.

### 106 REDUCING SUGARS AS INVERT SUGAR—OFFICIAL

(**a**) *Before inversion.*—Proceed as directed under **36** or **38**, using an aliquot of the soln used for direct polarization, **105** (**a**), and only neutral Pb-acetate for clarification.

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* See note 7, p. xvii.

(**b**) *After inversion.*—Proceed as directed under **36** or **38**, using an aliquot of the soln used for the invert polarization, **105** (**b**), and only neutral Pb-acetate for clarification.

## Sucrose

**107**

### POLARIMETRIC METHODS—OFFICIAL

*Sucrose in the Absence of Raffinose*

Proceed as directed under **22** or **23**.

**108**

### CHEMICAL METHODS

*By Reducing Sugars Before and After Inversion—Official*

Proceed as directed under **28**.

**109**

## Commercial Glucose—Official

Proceed as directed under **29**.

**110**

## Ash—Official

Heat 5 g of the sirup prepared as directed under **103** (**a**) (2) and **103** (**b**) (2) in a 50–100 cc Pt dish over a low flame until completely charred. Transfer to a muffle furnace and heat at low redness (not over 550°) until a white ash is obtained. If desired, interrupt the ashing when nearly all the C is burned, and after cooling add 0.5–1.0 cc of $H_2O$. Evaporate, and return the dish to the muffle. To the cooled ash add about 1 g of $(NH_4)_2CO_3$ free from non-volatile matter, and 0.5–1.0 cc of $H_2O$; evaporate to dryness; reheat in the muffle for 1–2 min.; cool in a desiccator; and weigh, taking precautions to guard against, or to correct for, absorption of moisture during weighing.

**111**

### SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under **12**.

**112**

### ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under **13**.

**113**

### ALKALINITY OF THE INSOLUBLE ASH—OFFICIAL

Proceed as directed under **14**.

**114**

### ALKALINITY OF TOTAL ASH—OFFICIAL

Add the alkalinities of the soluble and insoluble portions (**112** and **113**).

## Lead Number

### WINTON LEAD NUMBER[35]—TENTATIVE

**115**

### REAGENT

*Standard basic lead acetate soln.*—Boil 430 g of neutral Pb-acetate and 130 g of litharge for 30 min., or boil 560 g of dry basic Pb-acetate [**18** (**c**)] with 1 liter of $H_2O$. Cool, allow to settle, and dilute the supernatant liquid to 1.25 sp. gr. To a measured quantity of this soln add 4 volumes of $H_2O$ and filter if not perfectly clear. The soln should be standardized each time a set of determinations is made.

### 116 DETERMINATION OF LEAD IN THE BLANK

Transfer 25 cc of the standard basic Pb-acetate to a 100 cc flask, add a few drops of glacial acetic acid, and make up to the mark with $H_2O$. Shake, and determine $PbSO_4$ in 10 cc of the soln as directed under **117.** The use of acetic acid is imperative in order to retain all Pb in soln when the reagent is diluted with $H_2O$.

### 117 DETERMINATION

Transfer 25 g of the sample to a 100 cc flask by means of $H_2O$. Add 25 cc of the standard basic Pb-acetate soln and shake. Fill to the mark, shake, and allow to stand for at least 3 hours before filtering. Pipet 10 cc of the clear filtrate into a 250 cc beaker, add 40 cc of $H_2O$ and 1 cc of $H_2SO_4$, shake, and add 100 cc of 95% alcohol. Allow to stand overnight, filter on a weighed Gooch crucible, wash with 95% alcohol, dry in a water oven, and ignite in a muffle or over a Bunsen burner, applying the heat gradually at first and avoiding a reducing flame. Cool and weigh. Subtract the weight of $PbSO_4$ so found from the weight of $PbSO_4$ found in the blank, **116,** and multiply by the factor 27.33. The use of this factor gives the Pb number directly without the various calculations otherwise required.

## CANADIAN LEAD NUMBER[36] (FOWLER MODIFICATION)—TENTATIVE

### 118 REAGENT

*Standard basic lead acetate soln.*—Boil 280 g of dry basic Pb-acetate [**18** (**c**)] with 500 cc of $H_2O$. When soln is complete except for a slight sediment, pour off into a beaker or allow to cool in a dish and dilute with recently boiled $H_2O$ to a density of 1.25 at 20°.

### 119 DETERMINATION

Weigh the quantity of sirup containing 25 g of dry matter, transfer to a 100 cc flask, and make up to mark at 20°, or use the soln in which the conductivity value has been determined (**120**). Pipet 20 cc into a large test tube, add 2 cc of the standard basic Pb-acetate soln, cork, and allow to stand 2 hours.

Filter with suction on a 25 cc tared Gooch, having an asbestos mat at least 3 mm thick. When nearly all the liquid has run thru, fill the crucible with cold $H_2O$. Repeat to a total of 4 washings, taking care to prevent formation of fissures in the precipitate by keeping it covered with $H_2O$ and avoiding too great suction. Dry at 100°, weigh, and multiply the weight by 20.

## CONDUCTIVITY VALUE[37]—OFFICIAL, FIRST ACTION

### 120 DETERMINATION OF THE CELL CONSTANT

Prepare 0.1, 0.02, and 0.01 *M* KCl solns by dissolving respectively, 7.4560, 1.4912, and 0.7456 g of ignited purified KCl in $H_2O$ and diluting to 1 liter at 18°. Into a 100 cc beaker place 60 cc of the 0.01 *M* soln, insert a Van Zoeren or other dipping electrode, bring to 25°, and measure the electrical resistance. Multiply the number of ohms found by 141.2. Rinse the electrode and beaker with the 0.02 *M* soln, put in 60 cc of this soln, measure its resistance at 25°, and multiply by 276.8. Rinse the electrode with the 0.1 *M* soln, put in 60 cc of the soln, measure its resistance at 25°, and multiply by 1289. Average the 3 results (which should agree within 1%).

### 121 DETERMINATION

Weigh such a quantity of sirup as contains 25 g of dry matter. Transfer to a 100 cc volumetric flask with warm $H_2O$, cool to 20°, and dilute to the mark. Measure 60 cc of the soln into a 100 cc beaker, insert a Van Zoeren or other dipping electrode, bring to 25° (±0.1°), and measure the electrical resistance. Divide the constant of the cell by the observed number of ohms.

### 122 MALIC-ACID VALUE (COWLES)[38]—TENTATIVE

Weigh 6.7 g of the sample into a 200 cc beaker; add 5 cc of $H_2O$, then 2 cc of a 10% Ca-acetate soln; and stir. Add gradually and with constant stirring, 100 cc of 95% alcohol and agitate the soln until the precipitate settles, or let stand until the supernatant liquid is clear. Filter off the precipitate and wash with 75 cc of alcohol, 85% by volume. Dry the filter paper and ignite in a Pt dish. Add 10 cc of 0.1 *N* HCl [**II, 17** (**a**)] and warm gently until all the lime dissolves. Cool, and titrate back with 0.1 *N* NaOH soln, using methyl orange indicator. The difference in cc divided by 10 represents the malic acid value of the sample. Previous to use, the reagents should be tested by a blank determination and any necessary corrections applied.

### 123 METALS—TENTATIVE

Proceed as directed under **XXIX.**

## SUGAR BEETS

### SUCROSE

### 124 *I. Alcohol Extraction Method[39]—Tentative*

Pass the sample (usually in the form of cossettes) thru a meat grinder fitted with a plate having $\frac{1}{4}$-inch perforations and mix thoroly. Weigh 26 g of the beet pulp and transfer to a 100 cc flask with about 50 cc of 90% alcohol and 3–5 cc of basic Pb-acetate soln. Connect a reflux condenser to the flask and place on a boiling water bath for 10–15 min. Then pour the whole into a Soxhlet extractor, washing out the flask with fresh portions of 90% alcohol. Connect the same 100 cc flask to the extractor and fit the latter with a return condenser. Add 90% alcohol until the siphon is started and the flask is about $\frac{3}{4}$ full. Place the flask in a covered water bath kept at a temp. that will allow the alcohol to boil freely. Continue the extraction for 1–4 hours, or until a test of the alcohol in the extractor gives no color with alpha-naphthol soln when tested as follows: Introduce into a test tube a few drops of the alcohol coming from the extractor and add 4 or 5 drops of a 20% alcoholic alpha-naphthol soln and 2 cc of $H_2O$. Shake well, tip the tube, and allow 2–5 cc of colorless $H_2SO_4$ to flow down the side of the tube; then hold the tube upright and, if sucrose is present, a color varying from a faint to deep violet will be noted at the junction of the 2 liquids. On shaking, the whole soln becomes a blue violet color. This test is suitable for this work, but it must be remembered that other substances besides sucrose give this color reaction.

Remove the flask from the water bath; transfer the contents to a 100 cc volumetric flask; cool to the standard temp.; dilute to the mark with 90% alcohol; shake; and filter, keeping the funnel covered with a watch-glass. Polarize in a 200 mm tube.

Avoid evaporation and changes of temp. and use a minimum quantity of basic Pb-acetate for clarification, 3 cc rather than 5 cc. By digesting the beet pulp with the alcohol before extraction, the time of extraction is greatly shortened, the pulp becomes thoroly impregnated with the alcohol, all the air is removed, and a good extraction of the whole material is effected. If the pulp is fine and tends to clog the siphon, alcohol-washed cotton may be used as a plug in the extractor before adding the beet pulp, and a fine mesh screen may be placed over the pulp to keep the whole compact in the extractor.

### 125 *II. Hot Water Digestion Method I*[40]*—Tentative*

Weigh 26 g of the beet pulp, prepared as directed under **124**, and transfer with $H_2O$ to a wide-mouthed flask graduated to a content of 201.0 cc, the additional 1.0 cc representing an allowance for volume of marc and of Pb-precipitate; add 5–10 cc of basic Pb-acetate soln and sufficient $H_2O$ to produce a volume of about 160–170 cc; and shake. Immerse the flask in a water bath at 80° and rotate at intervals. At the end of 30 min. dilute to the mark with $H_2O$ at 80° and continue the digestion for 10 min. longer. Remove the flask from the water bath and allow it to cool to standard temp. Add sufficient strong acetic acid[41] to make the soln very slightly acid (generally less than 0.5 cc) and a few drops of ether to break the foam. Apply vacuum to withdraw retained air. Dilute to the mark at 20°, mix thoroly, filter, and polarize in a 400 mm tube.

### 126 *III. Hot Water Digestion Method II*[42] *– Tentative*

Use Ni-plated sheet iron vessels, 11 cm high, 6 cm body diameter, and 4 cm mouth diameter, also stoppers covered with tin foil to fit.

Weigh 26 g of the beet pulp, prepared as directed under **124**, on a watch-glass (small enough to go into the neck of the beaker) and transfer to the metal beaker; add 177 cc of dilute basic Pb-acetate soln [5 parts of basic Pb-acetate soln (sp. gr. 1.25) to 100 parts of $H_2O$]; shake; and stopper lightly. Submerge the beaker in a water bath at 75–80° for 30 min., shaking intermittently. When all the air has been expelled (generally after 5 min.), tighten the stopper. After 30 min., shake, cool to a standard temp., filter, add a drop of acetic acid to the filtrate, and polarize in a 400 mm tube. The reading is the percentage of sugar in the beet pulp.

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# XXXV. VEGETABLES AND VEGETABLE PRODUCTS[1]

## 1 PHYSICAL EXAMINATION—TENTATIVE

Note carefully the external appearance of the packages to detect the presence of "leakers," "swells," or "springers." In general, the ends of sound tins of canned vegetables are slightly concave. If desired, the vacuum or pressure in the can may be taken by a gage designed for that purpose.

Measure the distance from the top of the double seam to the contents of the can.[2]

Note particularly the odor of the vegetables, the appearance of the liquor or brine—whether clear or turbid—and the condition of the seams and the inner walls of the container, especially as to blackening and corrosion; note also the general appearance, color, flavor, and size of the vegetables. In all instances the analyst should familiarize himself with the normal appearance, odor, color, flavor, and other properties of the product under examination. Make careful macroscopic or microscopic examination for worm infestation, mold, dirt, or other evidence of decomposition or filth. If analysis of the gas in a bulged can is desired, the gas may be conveniently collected by means of the Doremus gas collector[3] or by similar apparatus.

## 2 PREPARATION OF SAMPLE—OFFICIAL

The preparation of the sample depends upon the character of the product and the determinations to be made. With samples composed of solid and liquid portions, proceed as follows: Weigh the full can, then open, make the physical measurements as directed under **1**, pour the entire contents on a round sieve with a No. 8 standard sieve (diameter of wire 0.84 mm and size of opening 2.38 mm).[4] Use a sieve 8 inches in diameter for a No. 3 or smaller can, and a sieve 12 inches in diameter for cans larger than No. 3.

Allow the can to drain 2 min., weigh either the drained solids or the free liquid direct, and reweigh the dry empty can. From the weights thus obtained determine the percentage of liquid and of solid contents. If only the solid portion is required for analysis or examination, thoroly grind the drained vegetables in a mortar or food chopper. If a composite of the solid and liquid portion is required, thoroly grind the entire contents of the can in a mortar or food chopper. In all cases, thoroly mix the portion used and preserve the balance in glass-stoppered containers. Unless the analysis is to be completed in a reasonably short time, determine the moisture in a portion of the sample prepared as above, and to prevent decomposition dry the remainder, grind, mix thoroly, and preserve in glass-stoppered containers. A second moisture determination is required in this procedure.

## 3 MOISTURE—OFFICIAL

Proceed as directed under **XXVII, 5.**

## 4 ASH—OFFICIAL

Proceed as directed under **XXVII, 8.**

## 5 SODIUM CHLORIDE—OFFICIAL

Determine Cl as directed under **XII, 33** or **35,** and express the result in terms of NaCl.

### 6 SUGARS—TENTATIVE

Determine reducing sugars and sucrose as directed under **XXVII, 21** and **22,** varying the weight of the sample used according to its sugar content.

### 7 TOTAL ACIDS—OFFICIAL

Proceed as directed under **XXVI, 26,** using 5 g of the sample. Express the result as the number of cc of normal alkali required to neutralize 100 g of sample.

### 8 VOLATILE ACIDS—OFFICIAL

Proceed as directed under **XVII, 46.** Express the result as percentage of acetic acid. 1 cc of 0.1 *N* alkali = 0.0060 g of acetic acid.

### 9 PRESERVATIVES—OFFICIAL

Proceed as directed under **XXXII.**

### 10 COLORING MATTERS—TENTATIVE

Proceed as directed under **XXI.**

### 11 METALS—TENTATIVE

Proceed as directed under **XXIX.**

## TOMATO PRODUCTS[5]

(Tomato catsup, pulp, purée, sauce, and paste)

### 12 PREPARATION OF SAMPLE—OFFICIAL

Shake the unopened container thoroly to incorporate any sediment; then transfer the entire contents to a large glass or porcelain dish and mix thoroly, continuing the stirring for at least 1 min. Transfer the well-mixed sample to a glass-stoppered container and shake or stir thoroly each time before removing portions for analysis.

### 13 TOTAL SOLIDS—TENTATIVE

Weigh 5 g of the sample into a flat-bottomed dish having a diameter of approximately 6 cm, distribute evenly in a thin layer over the bottom of the dish, evaporate to dryness on a steam bath, and dry at 70° for 4 hours at a pressure not to exceed 100 mm of Hg.

### 14 INSOLUBLE SOLIDS—TENTATIVE

Wash 20 g of the sample repeatedly with hot $H_2O$, centrifugalizing after each additon of $H_2O$ and pouring the clear, supernatant liquid thru a weighed triple paper filter on a Büchner funnel. After 4 or 5 washings transfer the remaining insoluble matter to the filter, dry for 2 hours at 100°, cool in a desiccator, and weigh rapidly. The filter used should be dried previously for 2 hours at 100°, cooled in a desiccator, and weighed. A cylinder 1–1¼ inches in diameter and 5–6 inches long is convenient for washing and centrifugalizing.

### 15 SOLUBLE SOLIDS—TENTATIVE

To obtain the percentage of soluble solids, subtract the percentage of insoluble solids from the percentage of total solids.

### 16 SAND—TENTATIVE

Weigh 100 g of the well-mixed sample into a 2–3 liter beaker, nearly fill the beaker with $H_2O$, and mix the contents thoroly. Allow to stand 5 min. and decant the supernatant liquid into a second beaker. Refill the first with $H_2O$ and again mix the contents. After 5 min. more decant the second beaker into a third and the first into the second; refill and again mix the first. Continue this operation, decanting from the third beaker into the sink until the lighter material is washed from the sample. Then collect the sand from the 3 beakers on a weighed Gooch crucible, dry, ignite, and weigh.

Attention is especially called to the fact that under "Sand" only the figure obtained by the above method should be reported. The results obtained by the determination of ash insoluble in HCl are not applicable to the determination of sand, as the sand is so unevenly distributed that reliable results can be obtained only by taking a larger sample than is possible in the determination of ash.

### 17 ASH—OFFICIAL

Evaporate 10 g of the sample to dryness on a water bath and ignite as directed under **XXVII, 8.**

### 18 ALKALINITY OF THE ASH—OFFICIAL

Proceed as directed under **XXVI, 9.** Express the result as the number of cc of normal acid required to neutralize the ash from 100 g of the sample.

### 19 SODIUM CHLORIDE—OFFICIAL

Determine Cl as directed under **XII, 33** or **35,** using a $HNO_3$ soln of the ash (cf. **XII, 33** and **35**). Calculate and report the result as percentage of NaCl.

### 20 REDUCING SUGARS BEFORE INVERSION—OFFICIAL

Weigh 20 g of the sample into a 200 cc flask, dilute with about 100 cc of $H_2O$, clarify with a slight excess of neutral Pb-acetate soln, dilute to the mark, and filter. Remove the excess of Pb with anhydrous $Na_2SO_4$ or with dry K oxalate. Filter, and determine reducing sugars as directed under **XXXIV, 38.** Express the result as percentage of invert sugar.

### 21 REDUCING SUGARS AFTER INVERSION—OFFICIAL

Transfer 50 cc of the filtrate obtained under **20** to a 100 cc flask, add 5 cc of HCl, and let stand overnight, as directed under **XXXIV, 23 (c).** Nearly neutralize with NaOH soln, cool, dilute to the mark, and determine reducing sugars in an aliquot as directed under **XXXIV, 38.** Express the result as percentage of invert sugar.

### 22 SUCROSE—OFFICIAL

Proceed as directed under **XXXIV, 28.**

### 23 TOTAL ACIDS—OFFICIAL

Proceed as directed under **XXVI, 26,** using 5 g of the sample. Express the result as percentage of anhydrous citric acid. 1 cc of 0.1 $N$ alkali $=0.0064$ g of anhydrous citric acid.

### 24 VOLATILE ACIDS—OFFICIAL

Proceed as directed under **XVII, 46,** using 25 g of the sample, increasing the quantity of $H_2O$ used for the distillation, and collecting a correspondingly larger quantity of distillate. Express the result as percentage of acetic acid. 1 cc of 0.1 $N$ alkali $=$ 0.0060 g of acetic acid.

### 25 FIXED ACIDS—OFFICIAL

Multiply the percentage of volatile acids, **24,** by 1.067 and subtract the product from the percentage of total acids, **23,** to obtain the percentage of fixed acids as citric acid.

## MICROANALYSIS OF TOMATO PULP, PURÉE, SAUCE, PASTE[6]—TENTATIVE

### 26 APPARATUS

(a) *Compound microscope.*—Equipped with good objectives and oculars, giving magnifications of approximately 90, 180, and 500 diameters. For convenience of use the lenses should be adjusted so as to be parfocal. A mechanical stage is highly desirable. It is essential that the combination giving the low magnification be capable of adjustment, so as to give an area of the field of view of 1.5 sq mm (a circle whose diameter is 1.382 mm). With the higher powers the working distances must be ample to allow the free use of the blood-counting cell.

(b) *Drop-in cross-ruled disk.*[7]—For estimating lengths of mold filaments, an ocular drop-in disk cross-ruled in sixths of the ocular diaphragm opening is desirable.

(c) *Blood counting cell.*—Ruled in the Thoma or the old Neubauer system of rulings. The so-called "improved" system of Neubauer is not suitable for this purpose.

(d) *Howard mold-counting cell.*[8]—Constructed like a blood-counting cell but with unruled central disk about 19 mm in diameter.

### 27 MOLDS

Clean the special Howard cell so that Newton's rings are produced between the slide and the cover-glass. Remove the cover and place a small drop of the well-mixed sample upon the central disk; using a knife blade or scalpel, spread the drop evenly over the disk, and cover with the glass so as to give an even spread to the material.

It is of the utmost importance that the drop be mixed thoroly and spread evenly, otherwise the insoluble matter, and consequently the molds, will be most abundant at the center of the drop. Avoid squeezing out the more liquid portions around the margin. Discard mounts showing uneven distribution. In a satisfactory mount Newton's rings should be apparent when finally mounted, and none of the liquid should be drawn across the moat and under the cover-glass.

Place the slide under the microscope and examine with such adjustment that each field of view covers 1.5 sq mm. This area, which is of vital importance, may frequently be obtained by adjusting the draw-tube in such a way that the diameter of the field becomes 1.382 mm. Where such adjustment by means of the draw-tube is not possible, it is sometimes necessary to have a mechanic make an accessory drop-in ocular diaphragm with the aperture accurately cut to the necessary size. The diameter of the area of the field of view can be determined by use of a stage micrometer, or by employing the rulings on the blood-counting cell. In order to use the latter method it is necessary to bear in mind that a square whose diagonal is 1.382 mm has sides of approximately 0.977 mm. Hence the mm scale on the blood-counting cell can be used by such adjustment that the circle of the field of view cuts off the necessary amount from each corner of the mm-ruled square. When the instrument is properly adjusted, the quantity of liquid examined per field is 0.15 cmm (0.00015 cc).

Observe each field, noting the presence or absence of mold filaments, and give the result as positive or negative. Examine at least 25 fields prepared from each of two or more mounts. No field should be considered positive unless the aggregate length of the mold filaments present exceeds approximately $\frac{1}{6}$ of the diameter of the field. Calculate the proportion of positive fields from the results of the examination of all the observed fields and report as percentage of fields containing mold filaments.

### 28 YEASTS AND SPORES

Fill a graduated cylinder with $H_2O$ to the 20 cc mark and add the sample till the level of the mixture reaches the 30 cc mark. Close the graduate, or pour the contents into an Erlenmeyer flask, and shake vigorously for 15–20 seconds. To assure thoroness the mixture should not fill more than $\frac{3}{4}$ of the container in which the shaking is performed. For tomato sauce or pastes, or products running very high in the number of organisms or of heavy consistency, use 80 cc of $H_2O$ with 10 cc or 10 g of the sample. In the case of exceptionally thick or dry pastes, it may be necessary to make an even greater dilution.

Pour the mixture into a beaker. Thoroly clean the counting cell so as to give good Newton's rings. Stir thoroly the contents of the beaker with a scalpel or knife blade and after allowing to stand 3–5 seconds remove a small drop, place it upon the central disk of the blood-counting cell, and cover immediately with the cover-glass, observing precautions to secure production of Newton's rings and avoiding the overflowing of the liquid so as to run in between the cover and supporting surfaces of the slide. Allow the slide to stand not less than 10 min. before beginning to make the count. It is customary to make the count with a magnification of about 180 to 220 diameters.

Count the number of yeasts and mold spores on $\frac{1}{2}$ of the ruled squares on the disk (this amounts to counting the number in 8 of the blocks, each of which contains 25 of the small ruled squares). The total number thus obtained equals the number of organisms in 1/60 cmm (1/60,000 cc) if a dilution of 1 part of the sample with 2 parts of $H_2O$ is used. If a dilution of 1 part of the sample with 8 parts of $H_2O$ is used, the number must be multiplied by 3. In making the counts, the analyst should avoid counting twice the organisms that rest on a boundary line between two adjacent squares.

### 29 BACTERIA

Determine the number of rod-shaped bacteria from the mounted sample used in **28**, but before examination allow the sample to stand not less than 15 min. after mounting. Use a magnification of about 500 diameters.

Count and record the number of bacteria having a length greater than $1\frac{1}{2}$ times their width in an area including 5 of the small sized squares. Count the number in 5 such areas, preferably 1 from near each corner of the ruled portion of the slide and 1 from near the center. Determine the total number of rod-shaped bacteria in the 5 areas and multiply by 480,000. This gives the number of this type of bacteria per cc. If a dilution of 1 part of the sample with 8 parts of $H_2O$, instead of 1 part of the sample with 2 parts of the $H_2O$, is used in making up the sample, then the total count obtained in the 5 areas must be multiplied by 1,440,000. The bacteria sometimes exhibit a slight motion and thus may present momentarily an end instead of a side view. For this reason it is necessary to keep them under observation long enough to establish their true character. Thus far it has proved impracticable to count the micrococci present as they are likely to be confused with other bodies frequently present in such products.

### 30 FIELD CORN IN CANNED MIXTURES OF FIELD AND SWEET CORN[9]—TENTATIVE

Empty the contents of the can (No. 2), or the representative equivalent portion of a larger can, into a large beaker and remove the liquor and debris from fragments of kernels by flotation with cold $H_2O$. Place upon a flat plate all kernels to which the outer seed coat is still attached, mix thoroly, and quarter to about 400 pieces. Harden the selected pieces in 95% alcohol and quarter again to obtain about 100 fragments. Cut each fragment thru with a section razor or knife and avoid contamination of the fragments with dextrin by washing and drying the instrument after each cut. With a dissecting needle remove a portion about $\frac{1}{16}$ inch in diameter from the uncontaminated interior of each kernel and place the pieces in separate depressions of a white spot plate. Cover each piece with I stain (0.2 g of I, 1.5 g of KI in 100 cc of $H_2O$) and allow to stand 10 min. A dense brown cloud will disseminate from the portions of sweet corn due to dextrin, while the soln surrounding the field corn will remain clear and the portion will be blue black and sharply outlined. Crush the field corn portions to insure absence of dextrin and count those found to contain none. Use care in interpreting the results because kernels of immature sweet corn do not contain enough dextrin to produce the dense brown coloration characteristic of more mature sweet corn. In case of doubt, report as field corn only those kernels having a firm texture. Calculate the percentage of field corn from the total number of kernels examined.

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# XXXVI. VITAMINS*

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* See note at bottom of p. xv.

# XXXVII. WATERS, BRINE, AND SALT

## WATERS

## POTABLE WATER

### TURBIDITY—OFFICIAL

1

#### REAGENTS

(**a**) *Standard turbidity soln.*—Mix 1 g of elutriated fullers' earth previously dried and sifted thru a 200-mesh sieve with $H_2O$ and dilute to 1 liter. If the fullers' earth is of good quality and the proper degree of fineness, this stock mixture has a turbidity of 1000. Check the stock soln with a turbidimeter equipped with either candle or electric light.

(**b**) *Turbidity standards.*—Prepared by dilution of (**a**).

2

#### DETERMINATION

If the turbidity is less than 100, which prohibits the use of the turbidimeter, determine by direct comparison with turbidity standards contained in bottles of clear white glass.

### COLOR—OFFICIAL

3

#### REAGENTS

(**a**) *Standard color soln.*—Dissolve 1.245 g of potassium chloroplatinate ($PtCl_42KCl$), containing 0.5 g of Pt and 1 g of crystallized $CoCl_2.\ 6H_2O$ containing 0.25 g of Co, in a small quantity of $H_2O$; add 100 cc of HCl; and dilute to 1 liter with $H_2O$. This stock mixture has a color of 500.

(**b**) *Color standards.*—Prepared by dilution of (**a**).

4

#### DETERMINATION

Compare the color of the sample, freed from suspended matter, with the standards in tubes of clear white glass.

5

### ODOR—OFFICIAL

Shake the vessel containing the sample and note the odor. Heat a portion of the sample to incipient boiling and note the odor.

6

### TOTAL SOLIDS—OFFICIAL

Thoroly shake the vessel containing the sample and pipet 100 cc of the unfiltered $H_2O$ into a weighed Pt dish. If the sample contains much suspended matter, shake; pour rapidly into a 100 cc measuring cylinder; and transfer without delay to a weighed Pt dish. Evaporate to dryness and heat to constant weight at 100°. In the case of highly mineralized waters it is advisable to weigh again after drying at 180°.

7

### SOLIDS IN SOLUTION—OFFICIAL

Allow the sample to stand until all sediment has settled and filter if necessary to secure a perfectly clear liquid. Occasionally a clear filtrate can be obtained only by the use of alumina cream but this should be avoided if possible. Evaporate 100–250 cc to dryness in a weighed Pt dish. Heat to constant weight at 100°. In the case of highly mineralized waters it is advisable to weigh again after drying at 180°.

### 8 SUSPENDED MATTER—OFFICIAL

(1) Subtract the value for solids in soln, **7**, from the value for total solids, **6**; or, (2) filter a suitable measured volume of the sample thru a dry weighed Gooch crucible containing an asbestos mat, dry crucible and contents at 100°, cool, and weigh.

### 9 IGNITED RESIDUE—OFFICIAL

Ignite the residue from **6** at a low red heat until the ash is white or nearly so. Note any odor or change in color produced during ignition. Record the weight of the ignited residue and calculate the loss on ignition.

## NITROGEN IN THE FORM OF FREE AND ALBUMINOID AMMONIA

### *Method I—Official*

(For waters that do not contain hydrogen sulfide)

### 10 REAGENTS

(a) *Sodium carbonate soln.*—Saturated.

(b) *Ammonia-free water.*

(c) *Standard ammonium chloride soln.*—1 cc = 0.01 mg of N in the form of $NH_3$.

(d) *Nessler reagent.**—Dissolve 143 g of NaOH in 950 cc of $H_2O$ and filter thru asbestos. Add 50 g of red $HgI_2$ to the filtrate and dilute with $H_2O$ to 1 liter. Mix thoroly, allow to settle, and use the supernatant liquid.

(e) *Alkaline potassium permanganate soln.*—Dissolve 143 g of NaOH and 8 g of $KMnO_4$ in $H_2O$ and dilute to 1 liter

### 11 DETERMINATION

Connect a flask of about 1000 cc capacity with an upright bulb condenser by means of a rather large glass tube and a soft rubber stopper or a recently extracted cork stopper. Place in the flask 5 cc of the saturated soln of $Na_2CO_3$ and 500 cc of $NH_3$-free $H_2O$. Distil into 50 cc Nessler tubes until no further traces of $NH_3$ are indicated on the addition of 2 cc of the Nessler reagent to 50 cc of the distillate. Continue the distillation until the volume of the soln in the flask has been reduced to about 200 cc. Cool slightly; add 500 cc of the sample under examination; and distil, at the rate of about 1 tubeful in 10 min., into 50 cc Nessler tubes until $NH_3$ ceases to be given off (4 or 5 tubes are usually sufficient). Add 2 cc of the Nessler reagent to each tube and let stand 10 min. Freshly prepare in a similar manner other nesslerized tubes containing known quantities of the standard $NH_4Cl$ soln, made up to 50 cc with $NH_3$-free $H_2O$, and compare the nesslerized distillates with these. Report as mg per liter of N in the form of free $NH_3$. Cool the flask and add 50 cc of the alkaline permanganate recently boiled. Distil, at the rate of 1 tubeful in 10 min., into 50 cc Nessler tubes until $NH_3$ ceases to come off. Nesslerize and compare as in the determination of free $NH_3$. Report as mg per liter of N in the form of albuminoid $NH_3$.

### *Method II—Official*

(For waters containing sulfur[1])

### 12 REAGENTS

(a) *Sulfuric acid soln.*—Dilute 7 cc of $H_2SO_4$ (free from $NH_3$-salts) to 500 cc.

(b) *Sodium carbonate soln.*—Dissolve 66 g of anhydrous $Na_2CO_3$ or 179 g of $Na_2CO_3 . 10\ H_2O$ in $H_2O$ and dilute to 250 cc.

The other reagents and solns used are described under **10**.

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* See note 7, p. xvii.

### 13 DETERMINATION

Place 500 cc of the sample in a beaker or casserole, add 30 cc excess of the $H_2SO_4$ soln **(a)** and boil carefully until free from sulfide (about 20 min.). Add about 300 cc of $H_2O$ and 8 cc of the $Na_2CO_3$ soln to a distilling flask connected as described under **11** and distil until free from $NH_3$. Cool, add the cooled sample, which has been freed from sulfide, and proceed with the distillation, addition of alkaline permanganate soln, etc., as directed under **11.**

## NITROGEN IN THE FORM OF NITRITE—OFFICIAL

### 14 REAGENTS

(a) *Sulfanilic acid soln.*—Dissolve 1 g of sulfanilic acid in hot $H_2O$, cool, and dilute to 100 cc.

(b) *Alpha-naphthylamine hydrochloride soln.*—Boil 0.5 g of the salt with 100 cc of $H_2O$, kept at constant volume, for 10 min.

(c) *Standard nitrite soln.*—Dissolve 1.1 g of $AgNO_2$ in $NO_2$-free $H_2O$, precipitate the Ag with NaCl soln, dilute to 1 liter, mix, and allow to settle. Dilute 100 cc to 1 liter and then 10 cc of this soln to 1 liter, using in each case $NO_2$-free $H_2O$. 1 cc of the last soln =0.0001 mg of N as $NO_2$.

### 15 DETERMINATION

Place 100 cc of the sample in a 100 cc Nessler tube and treat with 1 or 2 drops of HCl. Add 1 cc of the sulfanilic acid, 1 cc of the alpha-naphthylamine hydrochloride soln, and thoroly mix. Set aside for 30 min. with other Nessler tubes containing known quantities of the standard $NO_2$ soln made up to 100 cc with $NO_2$-free $H_2O$ and treated with HCl, sulfanilic acid, and alpha-naphthylamine hydrochloride soln in the same manner as the sample. Determine the quantity of $NO_2$ by comparing the depth of pink color in the known and unknown solns. Record as N in the form of $NO_2$.

## NITROGEN IN THE FORM OF NITRATE[2]

### *I. Phenoldisulfonic Acid Method—Official*

(For water of low chlorine content)

### 16 REAGENTS

(a) *Phenoldisulfonic acid soln.*—Dissolve 25 g of pure white phenol in 150 cc of $H_2SO_4$, add 75 cc of fuming $H_2SO_4$ (13–15% $SO_3$), and heat at 100° for 2 hours.

(b) *Standard nitrate soln.*—Dissolve 0.607 g of pure $NaNO_3$ in 1 liter of $NO_3$-free $H_2O$. Evaporate 50 cc of this soln to dryness in a porcelain dish; when cool, treat with 2 cc of the phenoldisulfonic acid soln, rubbing with a glass rod to insure intimate contact; and dilute to 500 cc. 1 =0.01 mg of N as $NO_3$. (This soln is permanent.) Prepare standards for comparison by adding $NH_4OH$ to measured volumes of the standard soln in 100 cc Nessler tubes.

(c) *Standard silver sulfate soln.*—Dissolve 4.397 g of $Ag_2SO_4$, free from $NO_3$, in 1 liter of $H_2O$. 1 cc =1 mg of Cl.

### 17 DETERMINATION

To 100 cc of the sample, or a quantity that contains 0.05 mg or less of N as $NO_3$, add the standard $Ag_2SO_4$ soln, precipitating all but about 0.5 mg of the Cl. Heat to boiling and allow to settle, or add a little alumina cream, filter, and wash with small quantities of hot $H_2O$. Evaporate the filtrate to dryness in a porcelain dish on a steam bath; when cool, treat with 2 cc of the phenoldisulfonic acid soln as directed under **16** (**b**). Dilute with $H_2O$ and add slowly $NH_4OH$ until the maximum color is developed. Filter if necessary, transfer to a colorimetric cylinder and compare with the standards in the usual manner. Record as N in the form of $NO_3$.

### *II. Reduction Method*[3]*—Official*

(For water of high chlorine content)

**18** REAGENTS

(**a**) *Aluminum foil.*—This reagent should be the purest obtainable. Cut into strips about 10 cm long, weighing about 0.5 g.

(**b**) *Sodium hydroxide soln.*—Dissolve 250 g of the purest NaOH obtainable in 1250 cc of $H_2O$. Add 2 or 3 strips of Al foil (**a**) and let stand about 12 hours. Concentrate the soln to 1 liter by boiling.

**19** DETERMINATION

To 100 cc of the sample, or a quantity that contains 0.1 mg or less of N as $NO_3$, in a 300 cc casserole, add 2 cc of the NaOH soln and concentrate by boiling to about ⅓ the original volume. Transfer to a 100 cc test tube, using N-free $H_2O$, and dilute, if necessary, to a volume of about 75 cc. Prepare a blank (preferably several blanks, since the N impurity in Al is often distributed unevenly) by placing about 75 cc of N-free $H_2O$ and 2 cc of the NaOH soln in a 100 cc test tube. Place a strip of Al foil in each tube. Close the ends of the test tubes with rubber stoppers connected by means of bent glass tubes with other test tubes containing about 50 cc of slightly acidified $NH_3$-free $H_2O$. (These latter tubes serve as traps to prevent the escape of $NH_3$ and at the same time permit the free evolution of H. Allow the sample and blank to stand at room temp. for 12 hours or until reduction is complete. Nesslerize the traps. If high in $NH_3$, indicating frothing over of the sample, discard the determination. Disregard the traps if they contain only 0.01–0.02 mg N as $NH_3$ each. Transfer the sample and blank to distillation flasks, using 250 cc of $NH_3$-free $H_2O$ for each; distil; nesslerize; and compare with standards as in the determination of free $NH_3$, **11**. Subtract the quantity of N found in the blank from that found in the sample. Calculate to mg per liter of N in the form of $NO_3$.

## CHLORIDE—OFFICIAL

**20** REAGENTS

(**a**) *Sulfuric acid.*—0.05 *N*.

(**b**) *Sodium carbonate.*—0.05 *N*.

(**c**) *Potassium chromate indicator.*—Dissolve 5 g of $K_2CrO_4$ in $H_2O$, add a soln of $AgNO_3$ until a slight permanent red precipitate is produced, filter, and dilute to 100 cc.

(**d**) *Standard silver nitrate soln.*—Dissolve 4.791 g of $AgNO_3$ in $H_2O$ and dilute to 1 liter. 1 cc = 1 mg of Cl. Check by titration against a standardized soln of NaCl.

**21** DETERMINATION

To 100 cc of the sample add a few drops of phenolphthalein indicator. If a pink color appears, titrate the $CO_3$ thus indicated to $HCO_3$ with 0.05 *N* $H_2SO_4$. If the sample is acid to methyl orange, add 0.05 *N* $Na_2CO_3$ to neutralize the acidity. Add 1 cc of the $K_2CrO_4$ indicator and titrate with the standard $AgNO_3$ soln. Correct for the quantity of $AgNO_3$ soln necessary to give, in 100 cc of Cl-free $H_2O$ with 1 cc of the chromate, the shade obtained at the end of the titration of the sample. (If iodides and bromides are found in interfering quantities, make the equivalent correction.)

If chlorides are present in very small quantities, concentrate 500 or 1000 cc in a porcelain dish to 100 cc, rub down the sides of the dish carefully, add 1 cc of the $K_2CrO_4$ indicator, and titrate with the standard $AgNO_3$ soln. If sufficient chlorides are present in 100 cc of the $H_2O$ to consume more than 25 cc of the standard $AgNO_3$ soln, determine by precipitation and weigh the AgCl as directed under **XII, 33.**

## OXYGEN REQUIRED

### *Method I—Official*

**22** REAGENTS

(a) *Standard potassium permanganate soln.*—Dissolve 0.395 g of $KMnO_4$ in 1 liter of $H_2O$. Each cc has 0.1 mg of O available for oxidation.

(b) *Standard oxalic acid soln.*—Dissolve 0.788 g of crystallized oxalic acid in 1 liter of $H_2O$.

Determine the value of the oxalic acid in terms of the permanganate by boiling 10 cc of the oxalic acid and 200 cc of redistilled $H_2O$ (prepared by treating distilled $H_2O$ with alkaline permanganate and distilling) with 10 cc of $H_2SO_4$ (1+3) and titrating, while still boiling, with the standard permanganate to the appearance of a pink color.

**23** DETERMINATION

Add 10 cc of $H_2SO_4$ (1+3) to 200 cc of the sample in a porcelain dish and heat to boiling. Add from a buret the standard permanganate until the $H_2O$ is distinctly red and boil for 10 min., adding more of the standard permanganate from time to time to maintain the red color. Add 10 cc of the standard oxalic acid and titrate back with the standard permanganate to a pink color. From the total number of cc of permanganate used, subtract the number of cc equivalent to 10 cc of the oxalic acid. The result is the number of cc of the permanganate required for 200 cc of the $H_2O$. Correct for sulfides, nitrites, and ferrous salts, if present, by subtracting the number of cc of the standard permanganate absorbed by another 200 cc portion of the sample when treated as above, except to make the digestion at room temp. and for a period of 3 min.

### *Method II*[4]*—Official*

(To be used when the chloride content of the sample is high)

**24** REAGENTS

(a) *Sodium hydroxide soln.*—Dissolve 50 g of NaOH in $H_2O$, cool, and make to 100 cc.

The other reagents and solns used are described under **22.**

**25** DETERMINATION

Introduce 100 cc of the $H_2O$ to be examined in a 300 cc flask, add 0.5 cc of the NaOH soln and 10 cc of the permanganate, boil for 10 min., allow to cool to 50–60°, and add 5 cc of $H_2SO_4$ (1+3) and 10 cc of the standard oxalic acid. As soon as the liquid has become perfectly colorless, and while constantly agitating, cautiously add the standard permanganate from a buret dropwise, until the liquid acquires a faint permanent redness. The permanganate used is the quantity required for decomposition of the organic matter in the 100 cc of the sample.

If 100 cc of the sample requires more than 4 cc of the permanganate for the oxidation of organic matter, make a second determination, using more of the permanganate and a correspondingly larger quantity of the NaOH, as undecomposed permanganate remaining after boiling must be at least twice as great as the quantity decomposed.

### DISSOLVED OXYGEN[5]

#### *Method I—Official*

(When more than 0.1 mg of nitrite nitrogen per liter is present)

#### **26** REAGENTS

(a) *Potassium permanganate soln.*—Dissolve 6.32 g of $KMnO_4$ in $H_2O$ and dilute to 1 liter.

(b) *Potassium oxalate soln.*—Dissolve 20 g of K-oxalate in $H_2O$ and dilute to 1 liter.

(c) *Manganous sulfate soln.*—Dissolve 480 g of $MnSO_4.4H_2O$ in $H_2O$ and dilute to 1 liter.

(d) *Alkaline potassium iodide soln.*—Dissolve 500 g of NaOH and 150 g of KI in $H_2O$ and dilute to 1 liter.

(e) *Sodium thiosulfate soln.*—0.025 *N*. Dissolve 6.205 g of chemically pure recrystallized $Na_2S_2O_3.5H_2O$ in $H_2O$ and dilute to 1 liter with freshly boiled and cooled $H_2O$. 1 cc = 0.2 mg of O or 0.1400 cc of O at 0° and 760 mm pressure. Inasmuch as this soln is not permanent it should be standardized occasionally against a 0.025 *N* soln of $K_2Cr_2O_7$.

(f) *Starch indicator.*—Prepare as directed under **VI, 3 (e)**.

#### **27** COLLECTION OF SAMPLE

Collect the sample in a narrow-necked glass-stoppered bottle of 250–270 cc capacity by means of an apparatus designed to avoid the entrainment or absorption of any O from the atmosphere. Note the temp.

#### **28** DETERMINATION

Remove the stopper from the bottle and add 0.7 cc of $H_2SO_4$ and then 1 cc of the $KMnO_4$ soln. Introduce these and all other reagents by pipet under the surface of the liquid. Insert the stopper and mix by inverting the bottle several times. If a noticeable excess of $KMnO_4$ is not present after 20 min., again add 1 cc of the permanganate soln; if this is still insufficient, use a stronger permanganate soln. After 20 min. destroy the excess of permanganate by adding 1 cc of the K-oxalate soln, re-stopper the bottle at once, and mix its contents. Add 1 cc of the $MnSO_4$ soln and 3 cc of the alkaline KI soln. Allow the precipitate to settle. Add 1 cc of $H_2SO_4$ and mix by shaking. Transfer 200 cc of the contents of the bottle to a flask and titrate with the 0.025 *N* $Na_2S_2O_3$, using a few cc of the starch indicator *toward the end* of the titration. Do not add the starch soln until the color has become faint yellow. Titrate until the blue color disappears. Report the results in mg per liter; if desired, report results also as percentage of saturation.[6]

#### *Method II—Official*

(When less than 0.1 mg of nitrite nitrogen per liter is present)

#### **29** REAGENTS

The reagents and solns used are described under **26.**

#### **30** COLLECTION OF SAMPLE

Proceed as directed under **27.**

31 DETERMINATION

Remove the stopper from the bottle and proceed as directed under **28**, beginning "Add 1 cc of the $MnSO_4$ soln."

## Lead, Copper, and Zinc[7]

(When present in small quantities)

### LEAD

*Method I—Tentative*

(Coloring matter, iron, lead, copper, and zinc present)

32 REAGENTS

(**a**) *Ammonium acetate soln.*—Dissolve 200 g of $NH_4$-acetate in $H_2O$ and dilute to 500 cc. The soln should be practically colorless.

(**b**) *Ammonium acetate soln.*—Dilute 50 cc of (**a**) to 500 cc.

(**c**) *Standard lead soln.*—Add $H_2SO_4$ in slight excess to a strong soln of Pb-acetate. Filter off the $PbSO_4$ and wash free from acid with $H_2O$. Dissolve the $PbSO_4$ in the $NH_4$-acetate soln, dilute to a definite volume, and determine the Pb as $PbCrO_4$ by precipitating with $K_2Cr_2O_7$ soln (cf. **VI, 51**). Dilute the stock soln so that 1 cc will contain 0.1 mg of Pb.

33 REMOVAL OF COLOR

Acidify 0.5–2 liters of the sample with HCl (1+1) and concentrate in a porcelain casserole to a volume of about 75 cc by heating slowly over an open flame. Add sufficient $NH_4Cl$ (about 2 g) to hold Mg in soln and assist in the separation of the sulfides. Add about 1 cc of $NH_4OH$ in excess and saturate with $H_2S$. Cover the dish, allow to stand about 2 hours, add more of the $NH_4OH$ and $H_2S$, boil a few minutes, let the precipitate settle, filter, and wash the precipitate once with hot $H_2O$. (The precipitate will contain all the Fe, Pb, Cu, and Zn, and the coloring matter will be in the filtrate.) Place the filter and precipitate in a small porcelain casserole, add 30 cc of $HNO_3$ (1+3), and boil. Filter, wash free from acid, and cool filtrate (soln A).

34 DETERMINATION

Add to soln A, under **33**, 5 cc of $H_2SO_4$ (1+1), evaporate nearly to dryness, and heat cautiously until copious fumes of $SO_3$ are given off. Cool, wash down the sides with a little $H_2O$, and repeat evaporation and heating. Transfer to a beaker with the aid of $H_2O$, add an equal volume of 95% alcohol, and let stand overnight. Filter off the $PbSO_4$; and wash with dilute alcohol, 50% by volume, until free from Fe. Collect the filtrate, which contains Fe, Cu, and Zn, in a 250 cc beaker (soln B). Digest the filter containing the $PbSO_4$ in a small porcelain casserole with about 40 cc of the warm $NH_4$-acetate soln, filter, and wash once or twice with the warm dilute $NH_4$-acetate soln and twice with $H_2O$. Dilute the filtrate to definite volume. To an aliquot portion add freshly prepared $H_2S$ water and a few drops of acetic acid (1+1). Compare the color obtained with a set of standards made by treating various quantities of the standard Pb soln with $H_2S$ water.

*Method II—Tentative*

(Coloring matter present; iron present to extent of 1 mg or less in quantity of sample taken for analysis; copper and zinc absent.)

### 35 REAGENTS

The reagents and solns used are described under **32.**

### 36 DETERMINATION

Remove coloring matter as directed under **33.** Add to soln A 5 cc of $H_2SO_4$ (1+1), evaporate nearly to dryness, and heat cautiously until copious fumes of $SO_3$ are given off. Cool, wash down the sides with a little $H_2O$, and repeat evaporation and heating. Transfer to a beaker with the aid of $H_2O$, add 25–40 cc of the $NH_4$-acetate soln, heat to boiling, and precipitate the Fe with $NH_4OH$. Filter and wash with the dilute $NH_4$-acetate soln and $H_2O$. Acidify the filtrate slightly with acetic acid (1+1) and determine Pb colorimetrically in the filtrate by the addition of freshly prepared $H_2S$ water as directed under **34.**

## *Method III—Tentative*

(Coloring matter, iron, copper, and zinc absent)

### 37 REAGENTS

The reagents and solns used are described under **32.**

### 38 DETERMINATION

Add 5 cc of $H_2SO_4$ (1+1) to 0.5–2 liters of the sample, evaporate nearly to dryness, and heat until copious fumes of $SO_3$ are given off. Transfer to a beaker with the aid of $H_2O$, add 25–40 cc of the $NH_4$-acetate soln, and determine Pb colorimetrically by the addition of $H_2S$ water as directed under **34.**

## 39 *Method IV—Tentative*

(Coloring matter absent; iron, lead, copper, and zinc present)

Add 5 cc of $H_2SO_4$ (1+1) to 0.5–2 liters of the sample, evaporate nearly to dryness, and heat until copious fumes of $SO_3$ are given off. Filter off the $PbSO_4$ and proceed as directed under **34.**

## COPPER—TENTATIVE

### 40 REAGENTS

**(a)** *Ammonium nitrate soln.*—Dissolve 10 g of $NH_4NO_3$ in $H_2O$ and dilute to 100 cc.

**(b)** *Potassium ferrocyanide soln.*—Dissolve 3.5 g of $K_4Fe(CN)_6 . 3H_2O$ in $H_2O$ and dilute to 100 cc. This soln should be freshly prepared.

**(c)** *Standard copper soln.*—Dissolve about 20 g of $CuSO_4 . 5H_2O$ in $H_2O$, add 1 cc of $H_2SO_4$, and dilute to 500 cc. Determine the Cu in 50 cc of this soln as CuO by precipitation with KOH soln. Dilute the stock soln so that 1 cc contains 0.1 mg of Cu.

### 41 DETERMINATION

Boil the moderately acid filtrate (soln B), which contains Fe, Cu, and Zn to remove alcohol; adjust the soln to a volume of about 200 cc; and add 1 g of $NH_4Cl$. Heat to boiling, saturate with $H_2S$ gas, and boil to remove precipitated S. Cover beaker, let stand about 2 hours or until supernatant liquid becomes clear, filter, and wash the CuS without intermission with $H_2O$ containing $H_2S$. Collect the filtrate in a porcelain casserole (soln C). Dissolve the precipitate of copper sulfide in hot $HNO_3$ (1+3). Cool, add a few drops of phenolphthalein indicator, and make the soln slightly alkaline with $NH_4OH$ added carefully from a dropping bottle. Add 10 cc of the $NH_4NO_3$ soln, adjust the volume to 100 cc, and boil gently until a test with red litmus paper shows the soln to be neutral. Filter the soln to remove any Fe that may be present and adjust the filtrate to a volume of 100 cc. To an aliquot add 3 drops of the $K_4Fe(CN)_6$ soln. Compare the color obtained with standards containing 0.1, 0.2, 0.3, 0.4, and 0.5 mg of Cu. Prepare these standards by measuring corresponding quantities of the standard Cu soln; adding phenolphthalein indicator, a slight excess of $NH_4OH$, and 10 cc of the $NH_4NO_3$; boiling the soln until neutral to red litmus, cooling, and adding 3 drops of the $K_4Fe(CN)_6$ soln. Make the colorimetric comparison in 100 cc Nessler jars.

### ZINC—TENTATIVE

**42**

#### REAGENTS

(a) *Citric acid soln.*—Dissolve 50 g of citric acid crystals ($C_6H_8O_7.H_2O$) in $H_2O$ and dilute to 100 cc.

(b) *Ammonium thiocyanate soln.*—Dissolve 20 g of $(NH_4)SCN$ in $H_2O$ and dilute to 1 liter.

(c) *Standard zinc*soln.*—Dissolve pure Zn in HCl and dilute so that 1 cc contains 0.1 mg of Zn.

(d) *Potassium ferrocyanide soln.*—Dissolve 3.5 g of $K_4Fe(CN)_6.3H_2O$ in $H_2O$ and dilute to 100 cc.

**43**

#### DETERMINATION

Boil the acid filtrate (soln C) from the copper sulfide precipitation to remove $H_2S$, cool, neutralize with $NH_4OH$, and add 10 cc of the citric acid soln. Heat to boiling and if no Ca-citrate separates add small quantities of powdered $CaCO_3$ until a precipitate of about 1 g of Ca-citrate is formed. Pass $H_2S$ thru the soln until it is cool. Let stand several hours, part of the time on a steam bath, until the supernatant liquid is clear.

Filter, wash with the $(NH_4)SCN$ soln and dissolve the precipitate on the filter with hot HCl $(1+9)$. If the filtrate is reddish in color, reprecipitate the Zn as before. Dispel turbidity of the filtrate due to colloidal S by boiling. When the filtrate is clear and colorless, dilute an aliquot to 45 cc in a 50 cc Nessler jar. Add 5 cc of the $K_4Fe(CN)_6$ soln, mix quickly, and compare the turbidity with standard Zn solns by viewing longitudinally the jars held over a sheet of fine print. Prepare the standards by mixing definite volumes of the standard Zn soln, 3 cc of HCl, $H_2O$ to make 45 cc, and 5 cc of the $K_4Fe(CN)_6$ soln. The unknown soln should contain a volume of acid equivalent to that in the standards. Do not use Zn borosilicate glassware in this determination.

## MINERAL WATER

### 44 SPECIFIC GRAVITY—OFFICIAL

Determine specific gravity at 20/4°* by means of a pycnometer (cf. **XVII, 24**).

### 45 SOLIDS IN SOLUTION—OFFICIAL

Determine as directed under **7**.

### 46 IGNITED RESIDUE—OFFICIAL

Determine as directed under **9**.

### 47 NITROGEN IN THE FORM OF FREE AND ALBUMINOID AMMONIA—OFFICIAL

Determine as directed under **11** or **13**.

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* See note 9, p. xvii.

### 48 NITROGEN IN THE FORM OF NITRITE—OFFICIAL

Determine as directed under **15.**

### 49 NITROGEN IN THE FORM OF NITRATE—OFFICIAL

Determine as directed under **17** or **19.**

### 50 CHLORIDE—OFFICIAL

Determine as directed under **21.**

## HYDROGEN SULFIDE[8]—OFFICIAL

### 51 REAGENTS

(**a**) *Hydrochloric acid.*

(**b**) *Starch indicator.*—Prepare as directed under **VI, 3** (**e**).

(**c**) *Iodine soln.*—0.02 *N*. Dissolve 10 g of KI (free from iodic acid) in a liter flask, using as little $H_2O$ as possible. Add 2.54 g of resublimed I and dissolve by shaking. Dilute to the mark with $H_2O$. Standardize against a $Na_2S_2O_3$ soln that has been recently standardized against a $K_2Cr_2O_7$ soln.

(**d**) *Iodine soln.*—0.01 *N*. Mix equal volumes of Reagent (**c**) and boiled $H_2O$. Standardize against a $Na_2S_2O_3$ soln as directed under (**c**).

### 52 DETERMINATION

Transfer a quantity of the sample to a graduated vessel by means of a siphon and add a few drops of phenolphthalein indicator. If alkaline, add HCl until the pink color of the indicator disappears. Add starch indicator, and with careful stirring titrate with I soln, (**c**) or (**d**), until a permanent blue color appears. Correct for the quantity of I soln needed to give an equally blue color. From the corrected quantity of I soln used, calculate the approximate quantity of $H_2S$ present. For accurate determinations siphon 100–500 cc of the sample, according to the quantity of $H_2S$ present, into a graduated vessel, keeping the outlet of the siphon below the liquid. Add immediately a sufficient quantity of HCl, calculated from the approximate determination, to make neutral to phenolphthalein indicator. Mix carefully with a bent glass rod and without delay add about 0.5 cc less I reagent (**c**) or (**d**) than is needed to combine with the $H_2S$ present.

Add 5 cc of starch indicator (**b**), and finish the titration with I soln dropwise with stirring until a blue color remains permanently. Correct for the quantity of I soln needed to give an equally blue color when the same quantity of starch soln is added to an approximately equal volume of boiled $H_2O$. If possible, make several determinations and take an average. Standardize reagents (**c**) and (**d**) frequently.

### 53 FREE CARBON DIOXIDE—TENTATIVE

If the sample reacts acid to phenolphthalein and alkaline to methyl orange, titrate 100 cc with 0.05 *N* $Na_2CO_3$ (free from bicarbonate) until the soln is neutral to phenolphthalein. The number of cc used multiplied by 1.1 gives the mg of free $CO_2$ in 100 cc. Express the results in mg per liter.

### 54 CARBONIC AND BICARBONIC ACIDS—OFFICIAL*

To 100 cc of the sample add a few drops of phenolphthalein, and if a pink color is produced titrate with 0.05 *N* HCl or $H_2SO_4$, adding a drop every 2–3 seconds until the pink color disappears. Multiply the buret reading by the factor 3 to obtain the mg of $CO_3$ ion in 100 cc. To the colorless soln from this titration, or to the original soln if no color is produced with phenolphthalein, add 1 or 2 drops of methyl orange; continue the titration without refilling the buret; and note the total reading. If

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* HCl has been substituted for $KHSO_4$, first action.

$CO_3$ is absent, multiply the total buret reading by the factor 3.05 to obtain the value of $HCO_3$ ion in mg per 100 cc. If $CO_3$ is present, multiply the reading with phenolphthalein by 2 and subtract from the total reading of the buret. Multiply the difference by 3.05 to obtain the $HCO_3$ ion in mg per 100 cc. Express the results as mg per liter.

## Silica, Iron, Aluminum, Calcium, Strontium and Magnesium

### 55 SILICA—OFFICIAL*

Make a preliminary examination, using 100–250 cc of the sample to determine the approximate quantity of Ca and Mg present, in order to ascertain the quantity of sample to be evaporated for the final analysis.

Evaporate the quantity sufficient to yield 0.1–0.6 g of CaO or 0.1–1 g of $Mg_2P_2O_7$ (usually 1–5 liters). Acidify the $H_2O$ with HCl and evaporate on a steam bath to dryness in a Pt dish. Continue the drying for about an hour. Thoroly moisten the residue with 5–10 cc of HCl. Allow to stand 10–15 min. and add sufficient $H_2O$ to bring the soluble salts into soln. Heat on a steam bath until soln of the salts is effected. Filter to remove most of the $SiO_2$ and wash thoroly with hot $H_2O$. Evaporate the filtrate to dryness and treat the residue with 5 cc of HCl and sufficient $H_2O$ to effect soln of the soluble salts, as before. Heat, filter, and wash thoroly with hot $H_2O$. Designate the filtrate as *A*. Transfer the 2 residues to a Pt crucible, ignite, heat over a blast lamp, and weigh. Moisten the contents of the crucible with a few drops of $H_2O$. Add a few drops of $H_2SO_4$ and a few cc of HF and evaporate on a steam bath under a hood. Repeat the treatment if all the $SiO_2$ is not volatilized. Dry carefully on a hot plate, ignite, heat over a blast lamp, and weigh. The difference between the 2 weights is the weight of the $SiO_2$. Add the weight of the residue to that of the total $Al_2O_3$ and $Fe_2O_3$ obtained under **56**. (If the residue weighs more than 0.5 mg, $BaSO_4$ may be found in it when Ba is present in the $H_2O$. If so, make the necessary correction and add to the weight of the total $Fe_2O_3$ and $Al_2O_3$ under **56**.)

### 56 IRON AND ALUMINUM—OFFICIAL

Concentrate *A*, under **55**, to about 200 cc; while still hot, add $NH_4OH$ slowly, with constant stirring, until alkaline to methyl orange. Boil, filter, and wash 2 or 3 times with hot $H_2O$. Dissolve the precipitate in hot HCl (1+1). Dilute to approximately 25 cc, boil and again precipitate with $NH_4OH$. Filter, wash thoroly with hot $H_2O$, dry, ignite, and weigh as $Al_2O_3$ and $Fe_2O_3$. (In the presence of $H_3PO_4$, the weight of this residue must be corrected for the $P_2O_5$ equivalent to the $H_3PO_4$ found under **70**, allowance being made for the difference in the volumes of the $H_2O$ used for these determinations.) Designate the filtrate as *B*.

### IRON

### 57 *Colorimetric Method—Official*

(If the quantity of iron is less than 1 mg)

Fuse in a Pt crucible the ignited precipitate of $Fe_2O_3$ and $Al_2O_3$ with fused $KHSO_4$, dissolve in $H_2O$, and precipitate the Fe and Al with $NH_4OH$. Dissolve the precipitate on the filter paper in HCl and $HNO_3$, dilute the soln, add 5% $(NH_4)SCN$ soln, and compare the color developed with that of calibrated color disks or standards containing known quantities of Fe.

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* The following changes have been made in the 2nd paragraph of the method as given in the 2nd Ed.: l. 4, 15 was changed to 10; l. 5, and l. 9, "dilute" was omitted; l. 5, "(1+1)" was deleted; l.8 "5–10" was changed to "5."

### 58 *Volumetric Method—Official*

Fuse in a Pt crucible the residue of $Fe_2O_3$ and $Al_2O_3$ with fused $KHSO_4$. This fusion takes but a few minutes and must not be continued beyond the time actually needed. When the fusion is completed, set the crucible aside and allow to cool. Add $H_2SO_4$ (1+4) and heat the crucible until the fused mass is dissolved. Evaporate on a steam bath as far as possible; then heat gradually until copious fumes of $SO_3$ are given off. Dissolve in $H_2O$ and allow to stand on the steam bath. Cool, transfer to an Erlenmeyer flask, and make up to such a volume that the soln does not contain more than 2.5% of free $H_2SO_4$. Pass $H_2S$ thru the soln to reduce the Fe and precipitate any Pt contaminating the residue from the fusion. (Zn may be used instead of $H_2S$ for reducing the Fe.) Filter, wash, and again pass $H_2S$ thru the soln so that all the Fe will be reduced. Expel the $H_2S$ by boiling, at the same time passing a current of $CO_2$ thru the soln. Test the escaping gas with Pb-acetate paper to ascertain the complete removal of $H_2S$. Discontinue boiling and let the flask cool without discontinuing the current of $CO_2$. Titrate the reduced Fe with a standard permanganate soln, 1 cc = 1 mg of Fe, and calculate as Fe.

### 59 ALUMINUM—OFFICIAL

To obtain the weight of $Al_2O_3$, in the absence of phosphates, subtract from the weight of $Fe_2O_3$ and $Al_2O_3$, under **56**, the Fe under **57** or **58**, calculated to $Fe_2O_3$. Calculate to Al.

### 60 CALCIUM—OFFICIAL

Concentrate *B*, under **56**, to 150–200 cc, and to this soln, containing an equivalent of not more than 0.6 g of CaO, or 1 g of $Mg_2P_2O_7$, add 1–2 g of oxalic acid and sufficient HCl (1+1) to clear the soln. Heat to boiling and neutralize with $NH_4OH$, stirring constantly. Add the $NH_4OH$ in slight excess and allow to stand 3 hours in a warm place. Filter off the supernatant liquid and wash the precipitate once or twice by decantation with 1% $NH_4$-oxalate soln. Dissolve the precipitate in HCl (1+1), dilute to 100–200 cc, add a little more oxalic acid, and precipitate as above. After allowing to stand 3 hours, filter, wash with the 1% $NH_4$-oxalate soln, dry, ignite, heat over a blast lamp, and weigh as CaO and SrO. Subtract from this weight, the weight of SrO equivalent to the Sr under **61**. The difference is the weight of CaO. Calculate to Ca. Designate the combined filtrates and washings as *C*.

As a check on the CaO, evaporate to dryness the filtrate from the $Sr(NO_3)_2$ under **61**, beginning with "Filter, and wash with ether-alcohol mixture, etc."; dissolve the $Ca(NO_3)_2$ in $H_2O$, precipitate as oxalate, filter, wash, ignite, and weigh as CaO.

### 61 STRONTIUM[9]—TENTATIVE

Dissolve the oxides under **60** in $HNO_3$ (1+1) and test with the spectroscope for Sr. If Sr is present, transfer the $HNO_3$ soln to a small Erlenmeyer flask. Evaporate nearly to dryness over a low flame and heat in an air bath at 150–160° for 1–2 hours after the $H_2O$ is evaporated. Break up the dried material with a stirring rod and add 10–15 cc of a mixture of equal parts of absolute alcohol and ether to dissolve the $Ca(NO_3)_2$. Cork the flask and allow to stand with frequent shaking for 2 hours or longer. Decant the soln thru a 5.5 cm filter, reserving the filtrate. Wash the residue several times by decantation with small portions of ether-alcohol soln. Dry the residue and the filter paper and wash the filter paper repeatedly with small portions of hot $H_2O$, collecting the filtrate in the flask containing the main portion of the $Sr(NO_3)_2$ residue. Add 1 or 2 drops of $HNO_3$ (1+1), evaporate, dry, pulverize, and treat with 10–15 cc of the ether-alcohol mixture. Cork the flask and let stand about 12 hours with occasional shaking. Filter, and wash with ether-alcohol mixture until a few drops of the filtrate evaporated on a watch-glass leave practically no residue. Dry the paper and precipitate. Dissolve the $Sr(NO_3)_2$ in a few cc of hot $H_2O$. Add a few drops of $H_2SO_4$ and then a volume of alcohol equal to the volume of the soln and allow to stand 12 hours. Filter, ignite, weigh as $SrSO_4$, and calculate to Sr. Test spectroscopically for Ca and Ba. If these elements are present, determine the quantity and make the necessary correction.

### 62 MAGNESIUM—OFFICIAL

Concentrate *C*, under **60**, to about 200 cc and add 2–3 g of $(NH_4)_2HPO_4$ and sufficient HCl (1+1) to clear the soln when all the $(NH_4)_2HPO_4$ is dissolved. [$Na_2HPO_4$ or $NaNH_4HPO_4$ may be used instead of the $(NH_4)_2HPO_4$.] When cold, make slightly alkaline with $NH_4OH$, stirring constantly. Add 1–2 cc excess of the $NH_4OH$ and allow to stand about 12 hours. Filter off the supernatant liquid and wash 3 or 4 times by decantation with a soln of $NH_4OH$ (1+10). Dissolve the precipitate in HCl (1+1), dilute to about 150 cc, add a little $(NH_4)_2HPO_4$, and precipitate with $NH_4OH$ as before. Allow to stand 6–12 hours, filter, wash free from chlorides with $NH_4OH$(1+10), ignite, heat over a blast lamp, and weigh as $Mg_2P_2O_7$. Calculate to Mg.

## Sulfuric Acid, Sodium, Potassium, and Lithium

### 63 SULFURIC ACID—OFFICIAL

Make a preliminary examination, using 100–250 cc of the sample to determine the approximate quantity of sulfates. (The alkali salts present can be approximated by calculating the quantity of Na necessary to combine with the excess of acids—HCl, $H_2SO_4$, and $HCO_3$—over the Ca and Mg.

Take a quantity (usually 1–5 liters) sufficient to yield not more than 1 g of $BaSO_4$ and not more than 0.5 g of mixed chlorides. Acidify with HCl (1+1), evaporate to dryness in a Pt dish, and remove $SiO_2$ by 2 evaporations as directed under **55**, using not more than 2 cc of HCl for the final soln. Combine the filtrate and washings from the $SiO_2$ determinations and concentrate to about 150–200 cc. Heat to boiling and precipitate with a slight excess of 10% $BaCl_2$ soln, added very slowly and with constant stirring. Cover and allow to stand on a steam bath about 12 hours. Filter, thoroly wash the precipitate of $BaSO_4$ with hot $H_2O$, dry, ignite over a Bunsen burner, and weigh.

If the content of sulfate in the sample is unusually large, proceed as far as the concentration of the $SiO_2$ filtrates, as directed above. Add 50 cc of HCl, heat to boiling, and precipitate with $BaCl_2$ soln as before. Evaporate to dryness, wash the precipitate repeatedly by decantation, and filter. Complete the washing of the precipitate, ignite, and weigh. Calculate to the $SO_4$ ion. Designate the filtrate as *E*.

### SODIUM, POTASSIUM, AND LITHIUM

*Amyl Alcohol Method—Official**

*Ether Alcohol Method*[11]*—Official*

### 64 PREPARATION OF THE MIXED CHLORIDES

Evaporate to dryness *E*, under **63**, in a Pt dish and ignite the residue to faint redness to remove all traces of $NH_4$-salts. Dissolve the residue in the dish in about 200 cc of $H_2O$ and precipitate with milk of lime or a saturated soln of $Ba(OH)_2$. Boil, allow to stand for 30 min., and filter off the insoluble $Mg(OH)_2$ and undissolved lime. Thoroly wash the precipitate with hot $H_2O$ and combine the filtrate and washings.

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* See note 7, p. xvii.

If the precipitate of Mg is large, dissolve it in a small quantity of HCl, evaporate to dryness, take up with $H_2O$, and precipitate as before. Concentrate the 2 filtrates and washings to 200–250 cc. Add $NH_4OH$ and sufficient solid $NH_4$-carbonate to precipitate the Ca and Ba. Allow to stand on a steam bath for 1–2 hours. Filter off the supernatant liquid, dissolve the precipitate in HCl, reprecipitate as above, and wash thoroly with hot $H_2O$. Evaporate the combined filtrates and washings to dryness and drive off the $NH_4$-salts by gentle heat. Treat the residue with $H_2O$, pass thru a small filter, using as little wash $H_2O$ as possible evaporate to a small volume, and again precipitate with 1 or 2 drops of $NH_4OH$ and 2 or 3 drops of saturated solns of $NH_4$-carbonate and $NH_4$-oxalate. If any precipitate appears, filter and repeat the process. Evaporate the filtrate to dryness and drive off all $NH_4$-salts by heating to faint redness in a Pt dish. Treat the residue with a little $H_2O$, filter into a small Pt dish, add a few drops of HCl (1+1), and evaporate to dryness. Dry in an oven, heat to faint redness, cool in a desiccator, and weigh the combined chlorides of K, Na, and Li. Repeat the heating to constant weight ($x$). Dissolve the mixed chlorides in hot $H_2O$, filter, and wash. Return the filter paper and residue to the dish, dry, ignite, and weigh ($y$). The difference between ($x$) and ($y$) is the weight of the mixed chlorides.

## 65 DETERMINATION

Dissolve the mixed chlorides, obtained as directed under **64**, in a minimum quantity of cold $H_2O$ (about 1.5 cc will be more than sufficient for 0.5 g of the salts), introducing the soln into a tall 200 cc beaker. Add 1 drop of HCl, and then add *gradually* 20 cc of absolute alcohol, dropping the alcohol into the center of the beaker (not on the sides) while rotating the soln. (The NaCl and KCl should be precipitated in a perfectly uniform granular condition.) In a similar manner, add 60 cc of ether (sp. gr. 0.716–0.717 at 25°) and allow the mixture to stand about 5 min. or until the precipitate is well agglomerated and the supernatant liquid almost clear, rotating the mixture occasionally during this period. Filter thru a weighed Gooch crucible into an Erlenmeyer flask by means of suction, using a bell jar arrangement, washing the beaker thoroly with a mixture of 1 part alcohol and 5 parts ether, and collecting all the precipitate on the Gooch with the aid of a rubber-tipped rod. After thoroly washing the precipitate on the Gooch, set the latter aside and rinse the funnel with alcohol-ether mixture to wash any adhering Li soln into the flask containing the filtrate. Evaporate the filtrate to dryness on a steam bath, using an air blast. Treat the residue with 10 cc of *absolute* alcohol, warming if necessary, so that practically all the residue dissolves. If a slight film remains on the bottom and sides of the flask, remove it with a rubber-tipped rod. Then, while rotating the soln in the flask, add 50 cc of ether (sp. gr. 0.716–0.717 at 25°), followed by *1 drop* of HCl. Allow to stand for 30 min., rotating the soln at frequent intervals. When the fine precipitate has agglomerated (only a very small quantity is usually precipitated), filter into a tall beaker by means of suction thru the Gooch crucible containing the first precipitate. Wash the combined precipitates with the ether-alcohol mixture, taking the same precautions as in the first precipitation. Dry the Gooch and its contents in an oven, ignite gently, cool, and weigh to obtain the combined weight of NaCl and KCl. Reserve crucible and contents for the determination of K.

Evaporate on a steam bath the ether-alcohol filtrate and washings containing the Li. Dissolve the residue in a little $H_2O$, add a slight excess of $H_2SO_4$ (1+1), and transfer to a weighed porcelain or Pt dish. Evaporate as far as possible on a steam bath and then gently ignite the residue over a flame. (By placing the dish on a triangle over an asbestos gauze and using a low flame, the soln can be evaporated without spattering.) Finally ignite carefully over a full flame, cool, and weigh. If charring has occurred, repeat the ignition with $H_2SO_4$. Calculate to Li, using the factor 0.1263.

Remove the KCl and NaCl from the Gooch crucible by washing with 25–50 cc of hot $H_2O$, using suction, and collecting the filtrate in a porcelain dish. Add sufficient Pt soln [**II, 41(b)**] to convert the KCl and NaCl to $K_2PtCl_6$ and $Na_2PtCl_6$ and evaporate to dryness. Treat the residue with 80% alcohol by volume, filter, and wash until the excess of $PtCl_4$ and $Na_2PtCl_6$ has been removed. Dry the filter and precipitate, dissolve the residue in hot $H_2O$, and transfer to a weighed Pt dish. Evaporate on a steam bath, dry for 30 min. in an oven at 100°, cool, and weigh as $K_2PtCl_6$. Calculate to KCl, using the factor 0.3067, and to K, using the factor 0.1609.

Determine the weight of NaCl by subtracting the weight of KCl from the weight of combined KCl and NaCl. Calculate to Na, using the factor 0.3934.

### BARIUM

(It is not necessary to look for barium if sulfate is present in an appreciable quantity unless the $H_2O$ contains a large quantity of bicarbonate or chloride, which may hold in soln a small quantity of both sulfate and barium.)

### *Gravimetric Method*[11]—*Official*

**66** REAGENTS

(**a**) *Ammonium dichromate soln.*—Dissolve 100 g of the salt in $H_2O$ and dilute to 1 liter.

(**b**) *Ammonium acetate soln.*—Dissolve 300 g of the salt in $H_2O$, neutralize with $NH_4OH$, and dilute to 1 liter.

(**c**) *Dilute ammonium acetate soln.*—Dilute 20 cc of (**b**) to 1 liter.

Reaction of acetate solns should be alkaline rather than acid.

**67** DETERMINATION

Acidify a 1–5 liter portion of the sample with HCl and concentrate to about 200 cc. (If a precipitate forms, filter it off and examine for Ba.) Add about 0.5 g of $NH_4Cl$ and precipitate the Fe and Al with $NH_4OH$. Boil, filter, and wash. To the filtrate add an excess of the $NH_4$-acetate soln, 10 cc, keeping the total volume about 200 cc. Heat to boiling and add, with stirring, about 5 cc of the $NH_4$-dichromate soln. Allow to settle and cool. Decant the clear liquid thru a filter and wash the precipitate by decantation with the dilute $NH_4$-acetate soln until the filtrate is no longer perceptibly colored (100 cc of wash soln). Place the beaker under the funnel, dissolve the precipitate on the paper with warm $HNO_3$ (1+1), using as little as possible, and wash the paper. Add a little more acid to dissolve the precipitate in the beaker, then $NH_4OH$ until the precipitate which forms no longer redissolves. Heat to boiling; add, with stirring, 10 cc of the $NH_4$-acetate soln and 2 cc of the $NH_4$-dichromate soln; allow to cool slowly; and wash the precipitate by decantation with the dilute $NH_4$-acetate soln. Dry the $BaCrO_4$, burn the filter separately, ignite moderately to constant weight, and weigh as $BaCrO_4$. Record as Ba, using the factor 0.54217.

**68** *Volumetric Method—Official*

Proceed as directed under **67** through "wash the precipitate by decantation with the dilute $NH_4$-acetate soln" (after the second precipitation). Then proceed as follows:

Dissolve the precipitate in about 10 cc of HCl (1+1) and hot $H_2O$. Wash the filter, dilute the soln to about 400 cc, and add about 50 cc of a freshly prepared 10% soln of KI. Mix carefully and titrate the liberated I after 3 or 4 min. with 0.1 $N$ $Na_2S_2O_3$ soln. 1 cc of 0.1 $N$ $Na_2S_2O_3 = 4.579$ mg of Ba.

### PHOSPHORIC ACID—OFFICIAL

#### 69 REAGENTS

The reagents and solns used are described under **II, 8.**

#### 70 DETERMINATION

Treat 500 cc of the sample, or a larger quantity if necessary, with about 10 cc of $HNO_3$, and evaporate in a porcelain dish nearly to dryness to drive off HCl. Treat the residue with $H_2O$ and filter if necessary. Add $NH_4OH$ to alkalinity and then just enough $HNO_3$ to restore acidity. Add some solid $NH_4NO_3$ and heat in a water bath at a temp. of 45–50°. Add the molybdate soln and keep at the above temp. for 30 min. If more than a trace of the yellow precipitate is present, filter and wash with recently boiled and cooled $H_2O$ until entirely free from nitric and molybdic acids. Transfer the precipitate and filter to a beaker, add a little $H_2O$, and beat the paper and contents to a pulp. Dissolve the yellow precipitate in a small quantity of the standard KOH, add phenolphthalein indicator, and titrate with the standard acid. From the data so obtained calculate the $PO_4$ ion to mg per liter.

## 71 Manganese, Iodine, Bromine, Arsenic, and Boric Acid

Evaporate 0.5–2 liters of the sample to dryness after the addition of small quantities of solid $Na_2CO_3$. Boil the residue thus obtained with $H_2O$, transfer to a filter, and wash thoroly with hot $H_2O$. Use the residue remaining on the filter for the determination of Mn. Dilute the alkaline filtrate to a definite volume and use for the determination of I, Br, As, and $H_3BO_3$.

### MANGANESE

### *I. Persulfate Method—Official*

#### 72 REAGENTS

(**a**) *Silver nitrate soln.*—Dissolve 2 g of $AgNO_3$ in $H_2O$ and dilute to 1 liter.

(**b**) *Ammonium persulfate.*

(**c**) *Standard manganous sulfate soln.*—Dissolve 0.2877 g of pure $KMnO_4$ in about 100 cc of $H_2O$, acidify the soln with $H_2SO_4$ (1+1), and slowly heat to boiling. Add slowly a sufficient quantity of a 10% oxalic acid soln to discharge the color. Cool and dilute to 1 liter. 1 cc of this soln = 0.1 mg of Mn.

#### 73 DETERMINATION

Dissolve the insoluble residue under **71** in an excess of $HNO_3$ (1+1), evaporate to dryness, treat with $H_2O$, and add about 1 cc of $HNO_3$ and a little of the $AgNO_3$ soln. If a precipitate of AgCl appears, add more of the $AgNO_3$ until all the Cl is precipitated. Add an excess of about 10 cc of the $AgNO_3$ soln for each Mg of Mn present in the sample. Filter, add 1 g of $NH_4$-persulfate to the filtrate, and place the beaker or flask containing the soln on a steam bath until a pink color develops (usually about 20 min.). Compare the color developed with standards similarly prepared by treating solns containing known quantities of the standard $MnSO_4$ with the dilute $HNO_3$, $AgNO_3$ soln, and $NH_4$-persulfate.

### *II. Bismuthate Method*[12]*—Official*

#### 74 REAGENTS

(**a**) *Nitric acid (1+4).*—Free from brown oxide of N by aeration.

(**b**) *Dilute sulfuric acid.*—Dilute 25 cc of $H_2SO_4$ acid to 1 liter with $H_2O$. Add enough $KMnO_4$ soln to color the dilute acid faintly.

(**c**) *Standard manganous sulfate soln.*—Prepare as directed under **72** (**c**). A soln of $KMnO_4$ may be used in place of the $MnSO_4$ soln. To prepare it dissolve 0.2877 g of $KMnO_4$ in $H_2O$ and dilute to 1 liter.

### 75 DETERMINATION

Remove Cl by 2 or more evaporations with $H_2SO_4$ (1+1) from a quantity of the sample that contains 1 mg or less of Mn. The residue obtained under **71** may be used in place of a fresh sample by dissolving it in an excess of $HNO_3$ (1+4), adding the dilute $H_2SO_4$, and removing Cl by 2 or more evaporations. In either case, volatilize the $H_2SO_4$ and ignite the residue at a low heat (less than 500°). Dissolve in 40 cc of $HNO_3$ (1+3), add about 0.5 g of the Na-bismuthate, and heat until the permanganate color disappears. Add a few drops of a soln of $NH_4$- or Na-bisulfite to clear the soln and again boil to expel oxides of N. Remove from the source of heat, cool to 20°, again add 0.5 g of the Na-bismuthate, and stir. When the maximum permanganate color has developed, filter thru an alundum or Gooch crucible containing an asbestos mat that has been ignited, treated with a 4% soln of $KMnO_4$, and washed with $H_2O$. Wash the precipitate with $H_2SO_4$ (1+9) until the washings are colorless. Transfer the filtrate to a colorimeter tube. Compare the color developed with standards similarly prepared by treating solns containing known quantities of the standard $MnSO_4$ with the dilute $HNO_3$, $NH_4$- or Na-bisulfite soln, and Na-bismuthate. The color may also be compared with that of standards prepared from the $KMnO_4$ soln by diluting portions of 0.2, 0.4, 0.6 cc, etc., of the permanganate soln with the dilute $H_2SO_4$ to the same volume as the filtrate.

## IODIDE AND BROMIDE—TENTATIVE

(This method is qualitative and approximately quantitative. For accurate quantitative methods for iodides and bromides, see **106, 109, 113,** and **117.**)

### 76 REAGENTS

(**a**) *Sodium hydroxide soln.*—Dissolve 10 g of NaOH in $H_2O$, cool, and dilute to 100 cc.

(**b**) *Sodium nitrite soln.*—Dissolve 2 g of $NaNO_2$ in $H_2O$ and dilute to 1 liter.

(**c**) *Carbon disulfide.*—Freshly purified by distillation.

(**d**) *Chlorine water.*—Saturated and freshly prepared.

### 77 DETERMINATION

Evaporate to dryness an aliquot of the alkaline filtrate under **71**; add 2–3 cc of $H_2O$ to dissolve the residue and enough 95% alcohol to make the percentage of alcohol about 90. This precipitates the chlorides. Heat to boiling, filter, and repeat the preceding soln and precipitation once or twice. Add 2 or 3 drops of the NaOH soln to the combined alcoholic filtrates and evaporate to dryness. Dissolve this last residue in 2–3 cc of $H_2O$ and repeat the precipitation with alcohol, heating and filtering. Add a drop of the NaOH to this alcoholic filtrate and evaporate to dryness. Dissolve this residue in a little $H_2O$; acidify with $H_2SO_4$ (1+5), using 3 or 4 drops in excess; and transfer to a small flask. Add 4 drops of the $NaNO_2$ soln and about 5 cc of the $CS_2$. Shake until all the I is extracted and filter off the acid soln from the $CS_2$. Wash the flask, filter, and contents with cold $H_2O$ and transfer the $CS_2$ containing the I in soln to a Nessler tube, using approximately 5 cc of the $CS_2$. In washing the filter, make the contents of the tube up to definite volume, usually 12–15 cc, and compare the color with that of other tubes containing known quantities of I dissolved in CS., Prepare these standard tubes by treating measured quantities of a soln of known $KI_2$ content as described above, beginning with "acidify with $H_2SO_4$ (1+5)."

Transfer separately the acid soln of the sample and the standards from which the I has been removed to small flasks. To the standards add definite measured quantities of a bromide soln of known strength, and to each of the flasks containing sample and standards add 5 cc of the $CS_2$. Add Reagent **(d)**, 1 cc at a time, shaking after each addition until all the Br is set free. (Avoid a large excess of the Cl, as a bromo-chloride may form and spoil the color reaction.) Filter off the $H_2O$ soln from the $CS_2$ thru a moistened filter, wash the contents of the filter 2 or 3 times with $H_2O$, and then transfer to a Nessler tube by means of about 1 cc of $CS_2$. Repeat this extraction of the filtrate twice, using 3 cc of $CS_2$ each time. The combined $CS_2$ extracts usually amount to 11.5–12 cc. Add enough $CS_2$ to the tubes to bring them to a definite volume, usually 12–15 cc, and compare the sample with the standards. If, when using this method near its upper limit, the quantities of $CS_2$ recommended do not extract all the Br, make one or two extra extractions with $CS_2$; transfer the extracts to another tube; and compare the color with some of the lower standards. Add the readings thus obtained to the others.

Results closely approximating the true values for I and Br can be obtained in a shorter time on most samples by omitting the extractions with alcohol and comparing the color of the $CS_2$ solns directly in the extraction flasks.

## ARSENIC

### *Method I—Official*

**78**

#### REAGENTS

**(a)** *Zinc, arsenic-free.*

**(b)** *Sulfuric acid (1+5), arsenic-free.*

**(c)** *Standard arsenious oxide soln.*—Dissolve 0.0132 g of pure $As_2O_3$ in $H_2O$ containing about 50 mg of $Na_2CO_3$ and dilute to 100 cc. 1 cc of this soln = 0.1 mg of As.

**79**

#### DETERMINATION

Evaporate to dryness an aliquot of the alkaline filtrate under **71.** Acidify with the dilute $H_2SO_4$ and subject to the action of the Zn and the $H_2SO_4$ in a Marsh-Berzelius apparatus.[13] Compare the mirror obtained with a mirror prepared from an $As_2O_3$ soln of known strength. Calculate to the $AsO_4$ ion.

### *Method II—Official*

**80**

#### REAGENTS AND APPARATUS

The reagents, solns, and apparatus used are described under **XXIX, 1** and **2.**

**81**

#### DETERMINATION

Take a portion of the alkaline filtrate under **71** that contains not more than 0.03 mg of $As_2O_3$. If the quantity taken is greater than 10 cc, evaporate the soln to about that volume on a steam bath. Transfer the soln into the generator of the apparatus described under **XXIX,** with the aid of about 10 cc of $H_2O$; add 20 cc of $H_2SO_4$ (1+2); and proceed as directed under **XXIX, 4,** beginning with "add 5 cc of the KI reagent."

**82**

## BORIC ACID—OFFICIAL

(Glassware containing boron must not be used in this determination.)

*Qualitative test.*—Evaporate to dryness a part of the alkaline filtrate under **71**, treat with 1–2 cc of $H_2O$, and slightly acidify with HCl (1+1). Add about 25 cc of 95% alcohol, boil, filter, and repeat the extraction of the residue. Make the filtrate slightly alkaline with NaOH soln and evaporate to dryness. Add a little $H_2O$, slightly acidify with the dilute HCl, and place a strip of turmeric paper in the liquid. Evaporate to dryness on a steam bath and continue the heating until the turmeric paper is dry. If $H_3BO_3$ is present, the turmeric paper takes on a cherry-red color. As a confirmatory test, apply a drop of dilute $NH_4OH$ to the reddened paper. A dark olive color will be due to boric acid (cf. **XXXII, 15**).

*Quantitative method.*—If necessary to determine $H_3BO_3$ quantitatively, use the Gooch method.[14]

### 83 METHOD OF REPORTING RESULTS IN WATERS AND BRINE[15]—TENTATIVE

Report radicals and anhydrous salts in terms of mg per liter or, in the case of highly concentrated waters, in terms of g per liter. For the benefit of physicians, in the case of medicinal waters, report also the salts in terms of grains per quart, using the factor 0.014600 to convert mg per liter to grains per quart. In reporting salts in terms of grains per quart, convert the salts that have water of crystallization to the hydrated form as expressed in the U. S. Pharmacopoeia and in the National Formulary, and convert the $Mg(HCO_3)_2$ to $MgCO_3$ and $Ca(HCO_3)_2$ to $CaCO_3$. Use the following factors in these calculations:

$$\begin{aligned}\text{From } Na_2SO_4 \times 2.2682 &= Na_2SO_4.10H_2O.\\ MgSO_4 \times 2.0476 &= MgSO_4.7H_2O.\\ CaSO_4 \times 1.2647 &= CaSO_4.2H_2O.\\ Mg(HCO_3)_2 \times 0.5762 &= MgCO_3.\\ Ca(HCO_3)_2 \times 0.6174 &= CaCO_3.\end{aligned}$$

When a complete analysis is made report the error of analysis and state how it is distributed. Report only significant figures.

Report Fe and Al together when present in unimportant quantities and in calculations consider them as Fe. When Fe and Al are present in larger quantities, make the separation and report each separately.

In calculating the hypothetical combinations of acid and basic ions, join $NO_2$, $NO_3$, $BO_2$, and $AsO_4$ to Na; I and Br to K; and $PO_4$ to Ca. Assign the residual basic ions in the following order: $NH_4$, Li, K, Na, Mg, Ca, Sr, Mn, Fe, and Al, to the residual acid ions in the following order: Cl, $SO_4$, $CO_3$, and $HCO_3$. In case $HCO_3$ is not present in a sufficient quantity to join with all the Ca, the residual Ca is joined to $SiO_2$ to form $CaSiO_3$, and Mn, Fe, and Al are calculated to the oxides $Mn_3O_4$, $Fe_2O_3$, and $Al_2O_3$, respectively.

Use equivalent combining weights or their reciprocals in uniting the radicals, and when necessary for the purpose of comparison, in reducing salts to radicals and reuniting the radicals in the order specified above.

The equivalent combining weight of a radical is obtained by dividing its weight by its valence. The equivalent combining weight of a salt is obtained by dividing its molecular weight by the product of the valency of the basic element and the number of atoms of the basic element in the salt.

The procedure in calculating the hypothetical combinations by the use of the equivalent combining weights and their reciprocals is as follows:

Multiply the weights obtained, expressed in mg per liter, or, in the case of highly concentrated waters, in g per liter, for each radical to be combined, by the corresponding reciprocal of the equivalent combining weights. If the Na and K are to be determined by calculation, as is frequently the case, subtract the sum of the values obtained (reacting values) for the basic radicals from the sum of the reacting values for the acid radicals. The difference represents the reacting value of the undetermined Na and K. When all the constituents in the $H_2O$ have been determined the sums of the reacting values of the acid and of the basic radicals should be very nearly the same. In this case, if the difference is reasonable and well within the limit of accuracy of the methods employed, it may be distributed equally among all the radicals determined, or among those that the analyst believes to be less accurate than the others. If the difference is unreasonably great, repeat the analysis in whole or in part. The sums of the reacting values of the acid and basic radicals must be equal before proceeding with the calculation. Obtain the reacting values of the salts by subtracting in succession the reacting values of the radicals in the specified order. To convert these values to mg per liter of the respective salts multiply each of them by the equivalent combining weight of the respective salt.

## INDUSTRIAL WATER

### 84 SOLIDS IN SOLUTION—OFFICIAL

Determine as directed under **7**.

### 85 CHLORIDE—OFFICIAL

Determine as directed under **21**.

### 86 CARBONIC AND BICARBONIC ACIDS—OFFICIAL

Determine as directed under **54**.

### 87 NITRATES—OFFICIAL

Determine as directed under **17** or **19**.

### 88 SILICA—OFFICIAL

Determine as directed under **55**. Generally 1 evaporation with HCl for removal of $SiO_2$ is sufficient.

### 89 IRON AND ALUMINUM—OFFICIAL

Determine as directed under **56**.

### 90 CALCIUM—OFFICIAL

If no $H_3PO_4$ is present, concentrate the filtrate from the determination of Fe and precipitate with $NH_4OH$ and oxalic acid as directed under **60**. (Usually 1 precipitation is sufficient.)

### 91 MAGNESIUM—OFFICIAL

Determine as directed under **62**.

### 92 SULFURIC ACID AND ALKALIES—OFFICIAL

Proceed as directed under **63** and **65**. For technical purposes sufficient accuracy is obtained by determining the acids and the bases, except Na and K, and then calculating the excess of acid over basic ions to the Na salt, stating the alkali thus found as Na and K by difference.

### 93 TEMPORARY HARDNESS—OFFICIAL

The difference between the alkalinity after boiling, **97**, and the alkalinity before boiling, **95**, is the temporary hardness in parts per million of $CaCO_3$.

## ALKALINITY[16]

### I. Before Boiling—Official

94 REAGENTS

(a) *Sulfuric acid.*—0.02 *N*.

(b) *Erythrosine indicator.*—Dissolve 0.1 g of the Na salt in 1 liter of $H_2O$.

(c) *Chloroform.*—Neutral to erythrosine.

95 DETERMINATION

Measure 100 cc of the sample into a 250 cc white glass-stoppered bottle; add 2.5 cc of the erythrosine, 5 cc of the $CHCl_3$, and 0.02 *N* $H_2SO_4$ in small quantities, shaking the bottle vigorously after each addition of the acid. The rose color gradually disappears and is finally discharged by 1 or 2 drops of the acid. A white paper held back of the bottle facilitates the detection of the end point. Multiply the number of cc of 0.02 *N* $H_2SO_4$ used by 10 to obtain the number of parts per million of alkalinity in terms of $CaCO_3$.

### II. After Boiling—Official

96 REAGENTS

The reagents and solns used are described under **94**.

97 DETERMINATION

Boil 100 cc of the sample in a porcelain dish gently for 30 min. Cool, transfer to a 100 cc volumetric flask, and fill to the mark with recently boiled and cooled $H_2O$. Filter thru a dry paper and determine the alkalinity of the filtrate as directed under **95**, making the proper calculation for the aliquot employed and calculating in terms of $CaCO_3$ the parts per million of alkalinity after boiling.

## TOTAL HARDNESS[17]—OFFICIAL

98 REAGENTS

(a) *Soda reagent.*—Prepare an approximately 0.1 *N* soln, using equal parts of NaOH and $Na_2CO_3$.

(b) *Sulfuric acid.*—0.05 *N*.

99 DETERMINATION

Add sufficient 0.05 *N* $H_2SO_4$ to 200 cc of the sample contained in a 500 cc Pyrex or similar glass Erlenmeyer flask to neutralize the alkalinity, the quantity required being calculated from the results obtained as directed under **95**. Measure 200 cc of $H_2O$ into a similar flask. Treat the contents of each flask in the following manner: Boil 15 min. to expel free $CO_2$, add 25 cc of the soda reagent (**a**), boil 10 min., cool, rinse into 200 cc volumetric flasks, and dilute to 200 cc with boiled $H_2O$. Filter, rejecting the first 50 cc, and titrate 50 cc of each filtrate with the 0.05 *N* $H_2SO_4$ in the presence of methyl orange or erythrosine indicator. The total hardness in parts per million of $CaCO_3$ is equal to 50 times the difference between the cc of 0.05 *N* $H_2SO_4$ used in titrating the aliquot of the blank and the aliquot of the sample.

## 100 PERMANENT OR NON-CARBONATE HARDNESS—OFFICIAL

The difference between the alkalinity before boiling, **95**, and the total hardness, **99**, is the permanent or non-carbonate hardness expressed as parts per million of $CaCO_3$.

## IRRIGATING WATER

### 101 GENERAL METHODS—OFFICIAL

Determine the solids in soln: Cl, $CO_3$ and $HCO_3$, $H_2SO_4$, Ca, and Mg as directed under **7, 21, 54, 60, 62,** and **63,** respectively. To make the hypothetical combination, calculate Ca and Mg to the acid ions in the following order: $HCO_3$, $SO_4$, and Cl. Then calculate the remaining acid ions, including $CO_3$, to the corresponding salts of Na.

### BLACK ALKALI[18]—OFFICIAL

### 102 REAGENTS

(a) *Sodium carbonate.*—0.02 *N*. 1 cc = 0.00106 g of $Na_2CO_3$.

(b) *Carbon dioxide-free water.*—Boil $H_2O$ vigorously until approximately ⅓ of the original volume is evaporated, cool, and stopper.

The other reagents and solns used are described under **94.**

### 103 DETERMINATION

Transfer 200 cc of the sample to a Pt or Ag dish; add 50–100 cc of the 0.02 *N* $Na_2CO_3$, according to the quantity of soluble salts of Ca and Mg present; and evaporate to dryness. Rub up the residue with $CO_2$-free $H_2O$ and transfer to a 100 cc volumetric flask. (An ordinary laboratory wash bottle should not be used to transfer the residue, as the $CO_2$ from the breath of the operator is sufficient to vitiate the results.) Dilute to the mark, shake thoroly, and allow to stand until clear (12–15 hours). Remove 50 cc of the clear supernatant liquid, equivalent to ½ of the original quantity of sample and $Na_2CO_3$ added, and transfer to a 250 cc glass-stoppered flask or a stoppered titration bottle of clear glass, without any tinge of pink. Add 5 cc of the $CHCl_3$ and 1 cc of the erythrosine and titrate with the 0.02*N* $N_2SO_4$ until the color disappears. Shake the soln vigorously after each addition of the acid. The milky appearance produced by the $CHCl_3$ makes the reading of the end point sharp and certain.

(1) If less $H_2SO_4$ is required than is equivalent to ½ of the $Na_2CO_3$ added, due to some of the $Na_2CO_3$ reacting with soluble salts of Ca and Mg, the soln originally contained no black alkali in excess, but rather an excess of the so-called permanent or non-carbonate hardness. It is customary to express the hardness in terms of $CaCO_3$ or $CaSO_4$. With irrigating waters the latter form is to be preferred. Therefore, the difference between the number of cc of the $H_2SO_4$ required and ½ the number of cc of the $Na_2CO_3$ added, multiplied by the factor 0.00136, gives the equivalent of $CaSO_4$ in 100 cc of the sample.

(2) If more $H_2SO_4$ is required than that equivalent to ½ of the $Na_2CO_3$ added, black alkali was originally present in the soln, and the difference in cc, multiplied by the factor 0.00106, gives the black alkali in terms of $Na_2CO_3$ in 100 cc of sample.

## BRINE

### IODIDE IN THE PRESENCE OF CHLORIDE AND BROMIDE

### *Method I[19]—Tentative*

### 104 REAGENTS

(a) *Sodium hydroxide-sodium carbonate soln.*—Dissolve 50 g of a mixture of equal weights of NaOH and $Na_2CO_3$ in $H_2O$ and dilute to 1 liter.

(b) *Sodium hydroxide soln.*—Dissolve 4 g of NaOH in $H_2O$ and dilute to 100 cc.

(c) *Potassium permanganate soln.*—Dissolve 50 g of $KMnO_4$ in $H_2O$ and dilute to 1 liter.

(d) *Alcohol.*—95% by volume.

(e) *Potassium iodide crystals.*

(f) *Sodium thiosulfate soln.*—0.05 *N*. Dissolve 12.4 g of recrystallized $Na_2S_2O_3 \cdot 5H_2O$ in $H_2O$ and dilute to 1 liter.

**105** REACTIONS

$$KI + 2\ KMnO_4 + H_2O = KIO_3 + 2\ MnO_2 + 2\ KOH.$$
$$KIO_3 + 5\ KI + 6\ HCl = 6\ KCl + 3\ H_2O + 3\ I_2.$$
$$3\ I_2 + 6\ Na_2S_2O_3 = 6\ NaI + 3\ Na_2S_4O_6.$$

**106** DETERMINATION

Take a quantity of the brine that contains not more than 0.1 g of I nor more than 10 g of total salts. Adjust the volume to 100–150 cc, and add a sufficient quantity of Reagent (a) to precipitate the Ca and Mg. Boil, filter off the precipitate of Ca and Mg, and wash with hot $H_2O$. Introduce the filtrate into an Erlenmeyer flask, adjust the volume to about 100 cc, neutralize with $H_2SO_4$ (1+9), and add 1 cc of the NaOH soln. Heat to boiling; add an excess of the $KMnO_4$, about 0.5 cc excess; continue heating until the precipitate begins to coagulate; and allow to cool. Add sufficient 95% alcohol or $H_2O_2$ to bleach the permanganate color and set the beaker on a steam bath. When the precipitate has settled, filter and wash with hot $H_2O$. After cooling, add 1–2 g of KI, acidify with HCl, and titrate with the 0.05 *N* thiosulfate soln. Since one-sixth of the I titrated represents the quantity originally present, 1 cc of 0.05 *N* $Na_2S_2O_3$ soln = 1.058 mg of I.

### *Method II*[20]*—Tentative*

**107** REAGENTS

(a) *Chlorine water.*—Freshly prepared.

(b) *Potassium carbonate soln.*—Dissolve 100 g of $K_2CO_3$ in $H_2O$ and dilute to 1 liter.

(c) *Potassium iodide soln.*—Dissolve 200 g of KI, free from iodate, in $H_2O$ and dilute to 1 liter.

(d) *Sodium thiosulfate soln.*—0.05 *N*. Prepare as directed under **104** (**f**).

(e) *Starch indicator.*—Prepare as directed under **VI, 3** (**e**).

(f) *Starch iodide paper.*—Dip strips of filter paper in 25 cc of the starch indicator (e) that has been mixed with 5 cc of a 10% soln of KI.

**108** APPARATUS

(a) *Reaction flask.*—A glass-stoppered flask of 200–400 cc capacity provided with inlet and outlet tubes, the inlet tube having a stopcock and reaching nearly to the bottom of the flask and the outlet tube having a bulb of about 25 cc capacity blown near the center to lessen the danger of the absorbing soln being drawn back into the reaction flask.

(b) *Tall absorption bottle.*

**109** DETERMINATION

Reduce by evaporation a quantity of the sample that contains not more than 0.1 g of I to a volume of 25 cc, and place in the glass-stoppered reaction flask. Add 5 cc of the HCl (1+1), insert the stopper, and then add thru the inlet tube 50 cc of the freshly prepared Cl water. Place the end of the outlet tube in the tall absorption flask in which is placed 35 cc of the soln of $K_2CO$ diluted to 150 cc. Heat the reaction flask and boil gently until most of the Cl and Br has been distilled into the alkali. Connect the inlet tube to a $CO_2$ generator and complete the distillation by simultaneous boiling and bubbling of $CO_2$ thru the sample. Continue this for 10 min., testing for the presence of Cl and Br by holding a piece of starch iodide paper at the end of the outlet tube. Remove the source of heat and bubble $CO_2$ thru the apparatus until it is cool. Add 5 cc of the soln of KI and titrate the liberated I with the 0.05 *N* $Na_2S_2O_3$ soln.

### BROMIDE IN THE PRESENCE OF CHLORIDE BUT NOT IODIDE[21]—TENTATIVE

### 110 REAGENTS

(a) *Alkaline sodium sulfite soln.*—Dissolve 4 g of $Na_2SO_3$ and 0.8 g of $Na_2CO_3$ in $H_2O$ and dilute to 100 cc.

(b) *Chromium trioxide crystals.*

(c) *Hydrogen peroxide soln.*—3%.

(d) *Potassium iodide crystals.*

(e) *Sodium thiosulfate soln.*—0.05 *N*. Prepare as directed under **104** (f).

### 111 APPARATUS

(a) *Reaction cylinder.*

(b) *Two high-form Dreschel gas washing bottles.*

Join the reacting cylinder and the two gas washing bottles as shown in Fig. 36.

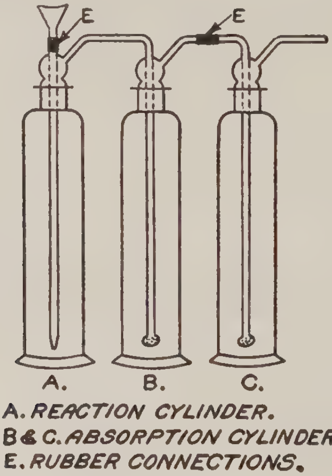

FIG. 36.—REACTION CYLINDER TO BE USED IN THE DETERMINATION OF BROMIDE. A—REACTION CYLINDER. B AND C—ABSORPTION CYLINDERS. E—RUBBER CONNECTIONS.

### 112 REACTIONS

$$2\ CrO_3 + 6\ HBr = Cr_2O_3 + 3\ H_2O + 3\ Br_2.$$
$$2\ H_2CrO_4 + 3\ H_2O_2 = Cr_2O_3 + 3\ O_2 + 5\ H_2O.$$
$$Na_2SO_3 + 2\ Br + H_2O = 2\ HBr + Na_2SO_4.$$

### 113 DETERMINATION

Take a quantity of the brine that contains not more than 10 g of total salts. The sample should not be too acid. Evaporate to dryness or nearly so. Charge reaction cylinder A by introducing glass beads to a depth of about 1 inch, followed by 15 g of the $CrO_3$ crystals and finally enough glass beads to fill the cylinder half full. Add 20 cc of the alkaline $Na_2SO_3$ soln to the first absorption cylinder and 5 cc to the second. Dilute each to about 200 cc. Connect the 3 cylinders and draw a current of air thru slowly. Wash the sample into the reaction cylinder with sufficient $H_2O$ to make about 25 cc of soln. Aspirate until the contents of the reaction cylinder are in soln and thoroly mixed, close the inlet tube with a small piece of rubber tubing and clamp, and reduce the pressure in the apparatus slightly by suction in order to guard against any possible escape of Br at the ground-glass stopper. Allow to stand overnight and then aspirate with a rather strong current of air (about 0.5–0.75 liter per min.) for 3 hours, adding four 2 cc portions of the 3% $H_2O_2$ to the reaction flask at 30 min. intervals. Stop the aspiration and evaporate the contents of the 2 absorption cylinders nearly to dryness. Clean out the reaction cylinder and freshly charge with glass beads and 15 g of the $CrO_3$. To the first absorption cylinder add 10 g of the KI crystals dissolved in 200 cc of $H_2O$ and to the second, 3 or 4 g in a like quantity of $H_2O$. Connect the apparatus; draw thru a slow current of air; and transfer the contents of the evaporating dish to the reaction cylinder by means of the small funnel, using 25 cc of $H_2O$. Aspirate until all the Br is evolved (about 1 hour) and titrate the KI soln with the standard $Na_2S_2O_3$ soln. 1 cc of 0.05 $N$ $Na_2S_2O_3 = 3.996$ mg of Br.

## BROMIDE IN THE PRESENCE OF CHLORIDE AND IODIDE—TENTATIVE

(Cooperative work indicates that the following is the best method that has been published for the determination of Br in the presence of Cl and I, but the results obtained show that only about 95% of the Br present is recovered when 80 mg of Br is contained in the portion of the sample taken for analysis. The method is satisfactory in the absence of I.)

### 114 REAGENTS

*Ferric sulfate crystals.*

The other reagents and solns are described under **110.**

### 115 APPARATUS

The apparatus and manner of connecting are described under **111.**

### 116 REACTIONS

$$Fe_2(SO_4)_3 + 2\ KI = 2\ FeSO_4 + I_2 + K_2SO_4.$$
$$2\ CrO_3 + 6\ HBr = Cr_2O_3 + 3\ H_2O + 3\ Br_2.$$
$$2\ H_2CrO_4 + 3\ H_2O_2 = Cr_2O_3 + 3\ O_2 + 5\ H_2O.$$
$$Na_2SO_3 + 2\ Br + H_2O = 2\ HBr + Na_2SO_4.$$

### 117 DETERMINATION

Introduce 10 cc of the sample into a distillation flask, adjust the volume to about 75 cc, and add 1.5–2.0 g of the $Fe_2\ (SO_4)_3.9H_2O$. Distil off the liberated I with steam, discarding the distillate. Transfer the residue from the distillation flask to a beaker, heat to boiling, add a few drops of methyl orange, and precipitate the Fe with $NH_4OH$, avoiding an excess of $NH_4OH$, as a precipitate of $Ca(OH)_2$ is bulky and difficult to wash. Filter off the $Fe(OH)_3$, wash with hot $H_2O$, and evaporate the filtrate and washings to dryness or nearly so, taking care that during the evaporation the soln does not become acid from hydrolysis of $MgCl_2$. From this point proceed as directed under **113**, beginning with "Charge reaction cylinder."

## SALT[23]

### 118 PREPARATION OF SAMPLE—TENTATIVE

If the sample is coarser than 20 mesh, grind so that all will pass thru a 20-mesh sieve, but avoid undue grinding so that as much as possible will be retained on an 80-mesh sieve. Mix sample by quartering and weigh all needed portions as nearly at the same time as possible.

### 119 MOISTURE—TENTATIVE

Place about 10 g of the sample in a dry, weighed Erlenmeyer flask of about 200 cc capacity. Weigh the flask and sample. Spread the sample evenly over the bottom of the flask by shaking gently and insert a small funnel in the neck. Heat the flask and sample for periods of 1 hour each on a triangle over the low, open flame of a gas stove at a temp. of about 250° until 2 consecutive weighings agree within 5 mg. Shake flask occasionally so that the sample will dry evenly. Report the loss of weight as moisture.

### 120 MATTERS INSOLUBLE IN WATER—TENTATIVE

Place 10 g of the sample in a 250 cc beaker, add 200 cc of $H_2O$ at room temp., and let stand 30 min., stirring frequently. Filter thru a weighed Gooch crucible with asbestos mat dried at 110°. Transfer the residue to the Gooch crucible with the aid of a rubber-tipped glass rod, using a total of not more than 50 cc of $H_2O$. Wash the residue with small portions of $H_2O$, about 10 portions of 10 cc each, until 10 cc of the filtrate shows only a faint opalescence upon addition of a few drops of $AgNO_3$ soln. Dry crucible and contents to constant weight at 110°. Call increase in weight of Gooch crucible, "matters insoluble in $H_2O$," and report results in percentage on a moisture-free basis. If matters insoluble in $H_2O$ exceed 0.1%, determine their nature.

### MATTERS INSOLUBLE IN ACID[24]—TENTATIVE

### 121 DETERMINATIONS

Treat 10 g of the sample with 200 cc of HCl (1+19), boil 2–3 min., and let stand 30 min., stirring frequently. Filter thru a Gooch crucible with mat, dried at 110°. Express results in percentage.

## Sulfate, Calcium, and Magnesium

### 122 PREPARATION OF SOLUTION—TENTATIVE

Weigh about 20 g of the sample, transfer to a 400 cc beaker, and dissolve in 200 cc of HCl (1+3). Cover beaker, heat to boiling, and continue boiling gently for 10 min. Filter thru paper filter and wash residue with small quantities of hot $H_2O$ until the filtrate is free from chlorides. Unite the filtrate and washings, cool, and make to a volume of 500 cc (soln *A*).

### 123 SULFATE—TENTATIVE

Place 250 cc of soln *A*, under **122**, in a 400 cc beaker of resistant glass, heat to boiling, and add a slight excess of a hot 10% $BaCl_2$ soln dropwise while stirring. Concentrate by heating gently and finally evaporate to dryness on a steam bath. Facilitate removal of free acid by stirring the partly dry residue. Wash the precipitate by decantation with small quantities of hot $H_2O$, finally transferring the precipitate to a close grained filter paper with the aid of a rubber-tipped glass rod and stream of hot $H_2O$. Wash the precipitate on the filter until the filtrate is free from chlorides. Test the filtrate for the presence of Ba. Dry and ignite the filter and precipitate over a Bunsen flame. Report the percentage of $SO_4$ in the sample on a moisture-free basis.

### 124 CALCIUM—TENTATIVE

Place the remainder of soln *A* in a 400 cc beaker of resistant glass. Add an excess of 10% oxalic acid soln (10 cc of the reagent usually will be sufficient). Add a few drops of methyl orange indicator and neutralize while hot by adding $NH_4OH$ dropwise, stirring constantly. Add about 1 cc excess of the $NH_4OH$, stir, and let stand in a warm place for 3 hours. Decant the supernatant liquid thru a filter, reserving the filtrate for the determination of Mg. Test the filtrate for Ca with $NH_4$-oxalate soln. Wash the precipitate in a beaker once with 10 cc of a 1% $NH_4$-oxalate soln, decanting thru filter paper. Combine filtrate and washings. Dissolve the precipitate in a beaker and on a filter with hot HCl (1+1), dilute to 100 cc, add a little more oxalic acid, and precipitate as before. After standing 3 hours, filter and wash with the $NH_4$-oxalate soln as before, reserving the filtrate and washings. Transfer the precipitate to a crucible, dry, ignite, and heat over a blast lamp to constant weight. Report as percentage of Ca on a moisture-free basis.

### 125 MAGNESIUM—TENTATIVE

Combine the filtrates and washings from the Ca determination, concentrate if necessary by boiling gently to a volume of about 150 cc, and proceed as directed under **62.** Report as percentage of Mg on a moisture-free basis.

### 126 METHOD OF REPORTING RESULTS IN SALT—TENTATIVE

(In the absence of added drying agents such as $MgCO_3$, Ca-phosphate, etc.)

Convert the sulfate to $CaSO_4$ and the unused Ca to $CaCl_2$, unless the sulfate in the sample exceeds the quantity necessary to combine with the Ca, in which case convert the Ca to $CaSO_4$ and the unused sulfate first to $MgSO_4$ and the remaining sulfate, if any, to $Na_2SO_4$. Convert the unused Mg to $MgCl_2$. Add the percentages of $CaCl_2$ and $MgCl_2$. Report on a moisture-free basis the percentage of matters insoluble in $H_2O$, of sulfate, of Ca, of Mg, of $CaSO_4$, and of $CaCl_2$ and $MgCl_2$. Report also the results of the qualitative examination of matters insoluble in $H_2O$, if the quantity exceeds 0.1% on a moisture-free basis.

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[23] J. Assoc. Official Agr. Chem., **5,** 384 (1922); **7,** 309 (1924).
[24] Ibid., **5,** 385 (1922); **6,** 129 (1923).

# XXXVIII. RADIOACTIVITY OF FOODS AND DRUGS—OFFICIAL, FIRST ACTION

## RADIUM IN CLEAR SOLUTIONS OF DRUGS AND WATERS[1]

### 1 REAGENTS

(a) *Sodium hydroxide soln.*—Dissolve 60 g of NaOH in $H_2O$, dilute to 3 liters, boil the soln at least 10 min., and keep hot until ready for use. Prepare the soln fresh each day.

(b) *Calcium chloride.*—Granular, anhydrous for drying.

(c) *Standard radium soln.*—Prepare a standard, dilute soln as follows: Measure accurately a quantity of the clear standard stock soln of radium that will contain about 5 millimicrograms of radium and place it in a flat-bottomed Erlenmeyer flask of about 200 cc capacity, provided with a sealed-in glass tube extending horizontally about 12 cm. If the volume of the standard soln taken is less than about 80 cc make it up to about 160 cc with a boiled soln of $HNO_3$ (1+12) containing about 0.1 g of $Ba(NO_3)_2$ per 100 cc. Record the exact quantity of standard soln taken and its temp. Boil the diluted soln rather vigorously for 20 min., carefully avoiding mechanical loss, allow to cool somewhat, replace the soln lost by evaporation with an equal quantity of boiled $H_2O$, and draw out and seal the end of the glass tube, recording the exact time of sealing. Allow the soln to stand at least 4 days.

### 2 APPARATUS

(a) *Gas collection apparatus.*—Two flat-bottomed long-necked Florence flasks, the one containing the sample having a capacity of 0.2–2 liters and the other a capacity of 2 liters (Nos. 1 and 2 in Fig. 37); 2 overflow flasks of about 300 cc capacity (3, 4); 1 gas-collecting buret 4 cm in diameter and 50 cm in length (5); 2 gas burners; and 2 iron supports provided with iron clamps, 1 iron tripod, sufficient rubber and glass tubing, wire gauzes with asbestos centers, and pinch cocks. Connect the apparatus as shown in Fig. 37.

(d) *Gas transfer apparatus.*—One gas transfer buret 4 cm in diameter and 50 cm in length (6); 1 separatory funnel with a capacity of 1 liter; 2 iron supports provided with iron clamps; rubber tubing, and pinch cocks.

(c) *Calcium chloride drying tube* (7).—A 1-hole rubber stopper and suitable glass and rubber tubing.

(d) *Control tube.*—A glass vial about 2 cm in diameter and about 6½ cm long (8), a 2-hole rubber stopper, and 2 glass tubes.

(e) *Alpha ray electroscope.*—A head, which is either permanently attached, as in Fig. 37, or detachable, as furnished by laboratory supply houses; an open discharge chamber; and a reading microscope (9) on a permanently attached base. The microscope contains a micrometer scale. The head of the electroscope contains a leaf system including the charging wire (10), and the open discharge chamber contains an adjustable support for the pan (11) and is provided with a door (12).

(f) *Emanation type electroscope.*—A head, which is either permanently attached, as in Fig. 37, or detachable, as furnished by laboratory supply houses; a gas-tight emanation chamber (13); and a reading microscope (14) on a permanently attached base. The microscope contains a micrometer scale. The head of the electroscope contains a leaf system consisting in part of a gold leaf and the charging wire (15). The gas-tight emanation chamber is provided with two stopcocks (16 and 17).

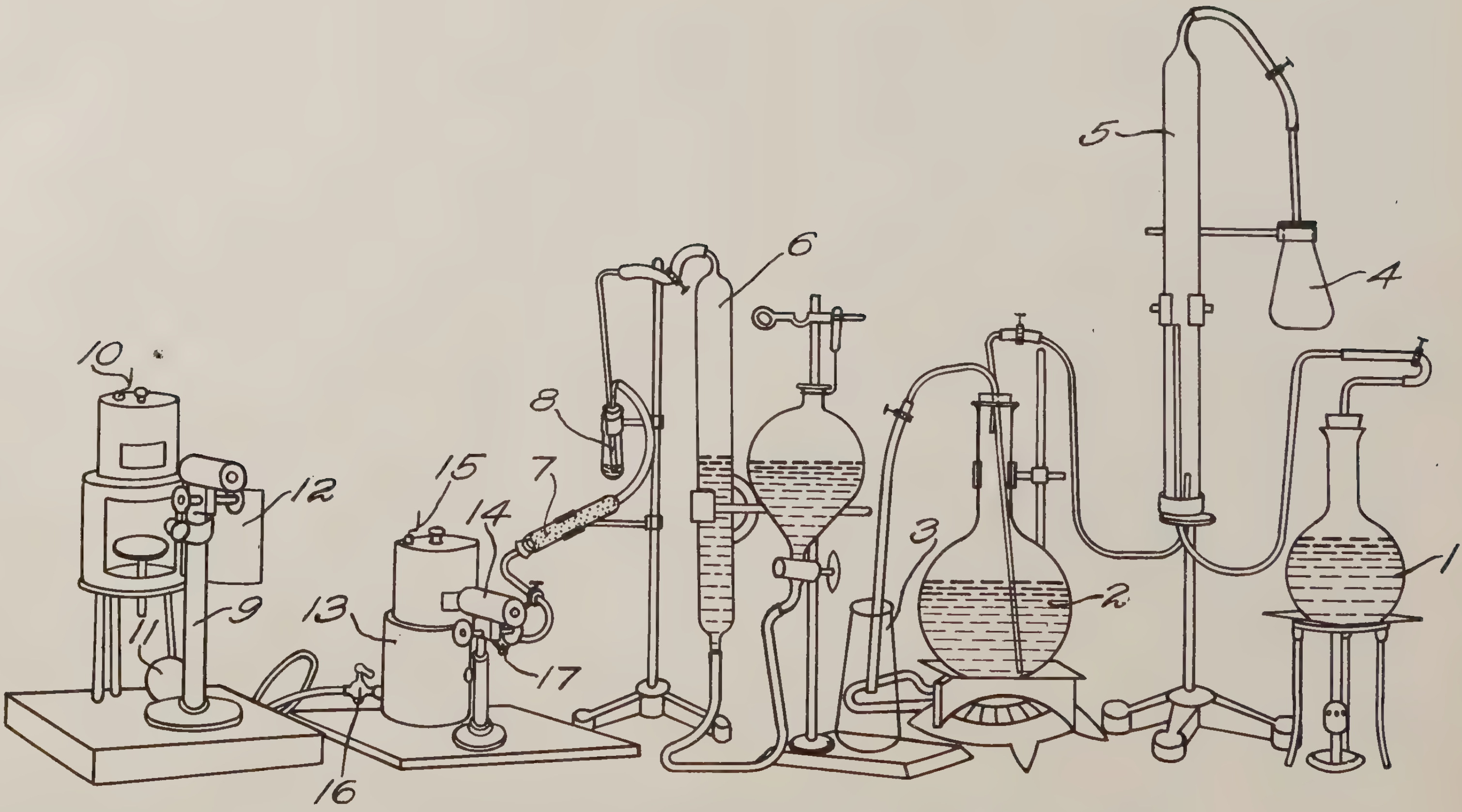
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FIG. 37.—APPARATUS FOR DETERMINING RADIOACTIVITY BY THE EMANATION METHOD

(g) *Charging device.*—The type furnished by laboratory supply houses or consisting of a hard rubber rod and catskin.

(h) *Stop watch.*

(i) *Vacuum pump.*

### 3 QUALITATIVE DETERMINATION

Charge the alpha ray electroscope thru the charging wire (10) to such an extent as to bring the leaf to a suitable position on the scale in the reading microscope (9). Close the door (12) and read the position of the leaf on the scale at frequent intervals, until the rate of fall of the leaf is constant. Calculate the average rate of fall of the leaf in divisions per min., designating the figure obtained as the natural leak of the instrument for that particular determination.

Place a convenient portion of the sample on the pan (11) and introduce it into the discharge chamber, close the door, recharge the leaf system, and record the rates of fall of the leaf in divisions per min. over the same range of scale as before, until the rate becomes constant, recharging if necessary. A rate of fall in excess of the natural leak of the instrument shows that the sample is radioactive.

## QUANTITATIVE DETERMINATION[2]

### 4 PREPARATION OF SAMPLE

*A. Samples completely soluble in acids:*

(**a**) If the sample is in solid or semi-solid form, add 50 cc of $HNO_3$ (1+9) and boil for several min. If a residue remains, add 50 cc of HCl (1+9) and again boil. (This treatment should not be applied to samples containing grease, such as face creams, the physical appearance of which will indicate that they are insoluble in aqueous solns.)

(**b**) If the sample is in liquid form, that is either a clear liquid, turbid liquid, or liquid containing suspended matter, add 50 cc of $HNO_3$ (1+9) to from 1 to 10 cc of it, boil for several min., and examine carefully for opalescence. If a portion of the sample remains undissolved, add 50 cc of HCl (1+9) and again boil. The addition of HCl to $HNO_3$ mixtures in this case and in the case of samples in solid and semi-solid form should be avoided if possible.

(**c**) If clear and limpid solns are obtained by the above procedures, take a quantity of the well-mixed sample that will produce an accurately measurable increase in the rate of fall of the leaf, dissolve as directed under (**b**), preferably in a Florence flask of 300 cc capacity, and proceed as under C "Final preparation of clear solns."

*B. Samples insoluble or incompletely soluble in acids:*

(**a**) *Preliminary treatment:*

(1) *Solids:* If the sample is not in powder form, grind to a fine powder, ignite a weighed portion of it in a porcelain dish in a muffle at dull red heat, avoiding fusion, and proceed as described below under (**b**) "Treatment of ash."

(2) *Semi-solids:* Ignite quite rapidly in a muffle a weighed portion of the sample contained in a porcelain dish, avoiding fusion. Heating too slowly or heating in the open may cause the sample to "creep" over the edge of the dish. Proceed as under (**b**) "Treatment of ash," below.

(3) *Liquids immiscible with $H_2O$:* Evaporate a weighted or measured portion of the sample to dryness, or as nearly so as possible, on a steam bath, and dry carefully on a hot plate. Ignite the residue in a muffle, avoiding fusion. Proceed as under (**b**) "Treatment of ash."

(4) *Liquids containing material which is insoluble in $HNO_3$* (1+9): Digest the sample or a suitable portion of it with $HNO_3$ (1+9). Filter into a 300 cc florence

flask, and wash the residue thoroly with hot $H_2O$. Proceed as under (b) "Treatment of ash," beginning "Ignite the washed residue in a Pt dish * * * ."

(b) *Treatment of ash:*

(1) Digest the ash obtained under (a) with $HNO_3$ (1+9) on a steam bath. Note the quantity of acid used in this and subsequent operations so that the final clear soln can be adjusted to contain about 10% of acid by volume. Filter into a florence flask and wash thoroly with hot $H_2O$. (Ordinarily a flask of 300 cc capacity is the most suitable, even if it is necessary to concentrate the filtrates by boiling.) Ignite the washed residue in a Pt dish and cover the residue with a few cc of $H_2O$ and 5–10 cc of HF. Evaporate to dryness on a steam bath. Add $H_2O$ and a few cc of $HNO_3$ (1+9), digest on a steam bath, filter into a florence flask, and wash with $H_2O$. Ash the filter paper in a Pt dish and add 5–10 cc of $H_2O$ and 1 cc of $HNO_3$. Examine carefully for any insoluble material, and if none is found, add the soln directly to the florence flask, rinsing out the Pt dish several times with $H_2O$ and adding the washings to the flask. Then follow the procedure under C "Final preparation of clear solns." If an insoluble residue remains, proceed as follows:

(2) Ignite the insoluble residue in a Pt dish and fuse with 5 to 10 times its weight of a fusion mixture consisting of equal weights of $K_2CO_3$ and anhydrous $Na_2CO_3$. After cooling, neutralize the fused mass with dilute $HNO_3$, using a drop of phenolphthalein soln to note when the soln is acid. Heat on a steam bath, add a few cc excess of $HNO_3$, and boil carefully. Filter the soln into the florence flask and wash thoroly. Ignite the insoluble residue in a Pt dish and proceed as under (b) "Treatment of ash," beginning with "cover the residue with a few cc of $H_2O$ and 5–10 cc of HF."

*C. Final preparation of clear solns:*

Boil the clear soln obtained under *A* or *B* for 20 min., and dilute with $H_2O$ and $HNO_3$ to a volume of about 250 cc if the sample is contained in a flask of 300 cc capacity; cool somewhat and seal without delay, noting exact time of sealing. All reagents should, of course, be free from Ra or radon. Seal the flask in the following manner: Bend a piece of glass tubing at right angles, draw out one end, and seal off. The sealed-off arm should be about 15 cm long and the other arm about 7 cm long. Place the short arm in a one-hole rubber stopper, so that the end is flush with bottom of stopper. Place stopper and tubing in neck of flask so that a tight fit is obtained and tie down to the flask with a cord. Examine the soln the next day. If an opalescence or precipitate is noted, filter the soln, wash the insoluble residue with hot $H_2O$, and treat it as described under *B* (2), beginning "Ignite the insoluble residue in a Pt dish and fuse . . . ."

## 5 STANDARDIZATION OF ELECTROSCOPE[1]

Determine the natural leak of the emanation type electroscope as follows: Create a vacuum in the emanation chamber (13) by means of the vacuum pump. Connect the closed emanation chamber with the freshly filled $CaCl_2$ drying tube (7) and with the glass vial (8), which should contain about 2 cc of $H_2O$. Allow dried air to enter the emanation chamber at the rate of one or two bubbles per second. When equilibrium is established, close off the emanation chamber by turning the stopcock (17), and illuminate the gold-leaf system and the micrometer scale in the reading microscope (14) by placing a small electric bulb behind the electroscope at such a distance that the electroscope will not be heated but the light will give a good illumination on the micrometer scale. Charge the electroscope thru the charging wire (15) to such an extent as to bring the gold leaf in view on a suitable part of the scale. Revolve the charging wire and ground it on the inner wall of the head of the electroscope.

After about 15–30 min., begin recording the exact positions of the gold leaf with respect to the scale, at intervals of about 10 min. for approximately an hour, noting the exact time and estimating to tenths of divisions. Calculate the average rate of fall of the gold leaf in divisions per min. and designate the figure obtained as the natural leak of the instrument for the particular determination.

Place about 1¾ liters of the 2% NaOH soln (Reagent **a**) in the 2-liter flask (2) and boil vigorously for at least 10 min. Lower the flame somewhat, manipulate the clips so as to fill the gas-collecting buret (5) with the soln, and continue boiling gently. Connect the Erlenmeyer flask containing the standard dilute Ra soln (Reagent **c**) with the buret, after scratching the capillary end of the glass tube with a file. Heat the sample gently and before excessive pressure is generated break off the capillary end inside the rubber connection, noting the exact time of breaking. Open the clip to permit evolution of gas into the buret. Continue heating the NaOH soln in the 2-liter flask (2) and boil the standard Ra soln rather vigorously for 20 min. Extinguish the flame under the sample and at the same time close the clip so as to retain the gases in the buret and disconnect the Erlenmeyer flask containing the standard Ra soln. Before the standard Ra soln is cold, draw out and seal off the glass tube of the Erlenmeyer flask containing it. Fill the gas transfer buret (6) with hot 2% NaOH soln that has been boiled for at least 10 min. and connect it with the upper end of the gas-collecting buret (5), so manipulating the clips as to transfer, without loss, the collected gases from the gas-collecting buret (5) to the transfer buret (6). Shut off the burner, disconnect the transfer buret from the gas-collecting buret, and connect it with the glass vial (8), the $CaCl_2$ drying tube (7), and the emanation chamber (13), in which a vacuum has been created. Perform this operation without loss of the collected gases. Allow these gases to pass into the emanation chamber at the rate of 1–2 bubbles per second. When the NaOH soln first enters the inlet tube of the glass vial, disconnect the gas transfer buret and allow the inflow of air to continue at the same rate until bubbling ceases. Close the stopcock (17) of the emanation chamber and disconnect the glass vial and the $CaCl_2$ drying tube. Allow the electroscope to stand 2¾ hours, charge it, and keep it charged for 15 min.; then begin a series of readings with the aid of a stop-watch on the same range of scale used in determining the natural leak of the electroscope. After the readings have been completed, draw air thru the emanation chamber for about 10 min. by means of the vacuum pump until the radon is removed. Subtract the natural leak of the instrument in terms of divisions per min. from the rate of fall in divisions per min. when the radon was in the electroscope. Divide the number obtained into the number of millimicrocuries of radon in the standard dilute Ra soln (Reagent **c**). (If the sample has been sealed for less than 30 days, calculate the radon content of the sample from a table of decay or growth of radon. If the sample has been sealed for 30 days or more, the radon content will be equivalent to the Ra content.) The quotient will be the quantity of Ra that will cause an acceleration of one division per min. in the rate of fall of the gold leaf. Repeat the standardization occasionally, using other dilute Ra solns prepared from the standard stock Ra soln.

## 6 PROCEDURE

Determine the natural leak of the electroscope, boil off the emanation from the sample, and determine its effect on the rate of fall of the gold leaf exactly as described previously under 5, taking the precaution of allowing the collected gases to remain in the gas-collecting or gas-transfer burets at least 10 min. for decay of any thoron that may be present. Subtract the natural leak in divisions per min. from the increased rate of fall, expressed in divisions per min., the difference being the rate of fall of the gold leaf due to radon in the sample. Multiply the figure obtained by the number of millimicrograms of Ra that will cause an increase of one division per min. The result will be the quantity of Ra in the sample if it has been prepared by boiling and sealing off and has been allowed to stand for at least 30 days. If the sample has stood for less than 30 days, calculate the Ra content from tables of decay and growth of radon. If the original sample is a soln, report the content of Ra in millimicrograms per cc or per liter. If the original sample is a solid, report the content of Ra in terms of millimicrograms per g or per 100 g.

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# XXXIX. DRUGS

### SAMPLING[1]—TENTATIVE

### *I. Tablets and Pills*

(**a**) *Bulk lots.*—Mix the lot as thoroly as possible without mutilating the contents. Count, weigh accurately, and grind thoroly at least 100 units. Calculate the average weight per unit.

(**b**) *Containers of 1000 or more units.*—Open and cautiously mix the entire contents without mutilation of the contents and divide into 2 parts, one of which is liberal for a thoro analysis; usually $\frac{1}{3}$ or $\frac{1}{4}$ is ample. Return the remainder to the container as a reserve sample. Accurately weigh, count, and grind the analyst's subdivision as in (**a**).

(**c**) *Containers of 100–500 units.*—If more than one container is available, weigh, count, and grind the entire contents of one of them. If only one container is available, but there is sufficient material to warrant subdividing, proceed as directed under (**b**); otherwise weigh, count, and grind the entire contents.

(**d**) *Small containers, such as tubes of hypodermic tablets.*—Choose a number of containers that will constitute a satisfactory analyst's sample; weigh, count, and grind the contents.

(**e**) *Tablets or pills of small dosages, for example, 1/100 grain of active ingredient.*—The number of units necessary may be so large as to render grinding unnecessary. An entire bottleful or half a bottleful may be required. Count the units to be employed and use them without grinding.

### *II. Soft Capsules*

Count and weigh a representative number of capsules and ascertain the gross weight per capsule. Open the capsules and transfer as much of the contents as possible to a weighing bottle. Deduct the weight of the cleaned, empty capsules from the gross weight and calculate the average net contents. To clean the gelatine capsules, cut in two if necessary and wash by agitating with alternate portions of alcohol and ether. Repeat until thoroly clean, finally removing the ether before a fan or air blast. A few drops of glacial acetic acid mixed with the alcohol aids in the cleaning.

### *III. Ampuls*

Before opening dislodge any liquid adhering in neck. Mark with a file or other suitable instrument the level of the liquid on the necks of the requisite number of ampuls, open them near the tip, transfer the bulk of the contents to a small flask, and mix. To determine the volume of contents, wash and dry the empty ampuls and fill them to the mark with $H_2O$ from a graduated pipet or buret.

### ACETANILID AND ACETPHENETIDIN[2] (PHENACETIN)

### 2 *Qualitative Test for Acetphenetidin—Tentative*

To 1–2 mg of the sample in a test tube add a drop of strong acetic acid, 0.5 cc of $H_2O$, and 1 cc of 0.1 $N$ I soln; warm the mixture to about 40°; and add a drop of HCl. If acetphenetidin alone is present, its periodide separates almost immediately in the form of reddish brown leaflets or needle-like crystals. If the sample consists largely of acetanilid, the separation takes place on cooling and shaking the liquid.

In the presence of considerable acetanilid, the periodide first separates as minute, oily globules, which on vigorous shaking gradually become crystalline. By this test as little as 0.5 mg of acetphenetidin, if alone, may be detected in the form of its characteristic periodide.

### *Quantitative Method—Tentative*

### 3 REAGENTS

(a) *Purified iodine.*—Dissolve 2 parts of resublimed I and 1 part of KI in 1 part of $H_2O$, pour the clear soln into a large volume of $H_2O$, filter, and wash the finely precipitated I several times on a perforated plate with water. Dry in the air and finally in a desicator containing $H_2SO_4$. It should be kept in a glass-stoppered weighing bottle.

(b) *Standard sodium thiosulfate soln.*—Dissolve 30 g of $Na_2S_2O_3.5H_2O$ in recently boiled, cooled $H_2O$ and dilute to about 1 liter. Standardize this soln against the purified I as follows: Weigh accurately about 0.3 g of the purified I in a small glass capsule provided with a closely fitting glass cap or stopper. Place the capsule in a 200 cc Erlenmeyer flask containing 0.5 g of KI dissolved in 1–2 cc of $H_2O$. After complete soln dilute with 10 cc of $H_2O$ and titrate with the $Na_2S_2O_3$ soln, using 1 or 2 drops of starch indicator [**VI, 3(e)**].

(c) *Standard iodine soln.*—Dissolve 40 g of KI in the least possible quantity of $H_2O$; add 30 g of I; and after soln dilute to about 1 liter. Standardize this soln against the standard $Na_2S_2O_3$ soln.

### 4 DETERMINATION

(a) *Acetphenetidin.*—(1) Place 0.2 g of the acetanilid-acetphenetidin mixture in a 50 cc lipped Erlenmeyer flask, add 2 cc of glacial acetic acid, heat gently over a wire gauze to complete soln, and dilute with 40 cc of $H_2O$ previously warmed to 70°. Transfer the clear liquid with two 10 cc portions of warm (40°) $H_2O$ to a glass-stoppered, 100 cc volumetric flask containing 25 cc of the standard I soln warmed to 40°. Stopper, mix thoroly by rotating the liquid, add 3 cc of HCl, continue rotating the liquid until crystallization begins, and then set aside to cool. (If the ratio of acetphenetidin to acetanilid is equal to or greater than unity, crystalline scales will form almost immediately on the addition of acid. As the proportion of acetanilid increases, however, the periodide tends to remain in the liquid state. Gentle agitation or rotation of the flask in $H_2O$, warmed not to exceed 40°, hastens the formation of crystals.) When the contents of the flask are at room temp., fill with $H_2O$ to within 2 or 3 cc of the mark; mix thoroly by rotating the mixture; and allow to stand overnight. Fill to the mark with $H_2O$; mix thoroly; allow to stand 30 min.; and filter thru a 5.5 cm dry, closely fitted filter into a 50 cc volumetric flask, rejecting about 15 cc of the first runnings but reserving it for the recovery of acetanilid. Transfer the 50 cc aliquot to a 200 cc Erlenmeyer flask and titrate with the standard $Na_2S_2O_3$ soln. Calculate the quantity of acetphenetidin from the following formula:

Acetphenetidin $= I\ (0.0896 \times N)$, in which

0.0896 = the number of g of acetphenetidin contained in 1 cc of a normal soln of this substance;
$N$ = the normality of the standard $Na_2S_2O_3$ soln used; and
$I$ = the number of cc of the standard $Na_2S_2O_3$ soln corresponding to the I combined with the acetphenetidin.

The formula of the precipitated periodide, which constitutes the basis for the above determination, is $(C_2H_5O.C_6H_4NH.COCH_3)_2HI.I_4$.

(2) The gravimetric determination of acetphenetidin may, if desired, be effected as follows: Filter off the periodide, preferably by suction; wash with 10–15 cc of the standard I soln; and transfer the precipitate, together with the filter and any particles of the precipitate remaining in the volumetric flask, to a separator, using not over 50 cc of $H_2O$. Remove both free and added I with a few small crystals of Na-sulfite and extract the liquid with three 50 cc portions of $CHCl_3$, washing each portion subsequently in a second separator with 5 cc of $H_2O$. After washing and clearing, filter the $CHCl_3$ soln thru a dry 5.5 cm filter into a 200 cc Erlenmeyer flask; distil most of the $CHCl_3$; transfer the residual soln (5–10 cc) by means of a little $CHCl_3$ to a small weighed beaker or crystallizing dish; evaporate to dryness on a steam bath; cool; and weigh.

(**b**) *Acetanilid.*—If the combined weight of the acetanilid-acetphenetidin mixture is known, determine the weight of the acetanilid by difference; or determine it directly from a second aliquot of the filtrate from the acetphenetidin periodide under (**a**) as follows:

Pipet 25–30 cc of the clear liquid into a separator; decolorize with solid Na-sulfite; add solid $NaHCO_3$ in slight excess; add 1 or 2 drops of acetic anhydride; extract with three 60 cc portions of $CHCl_3$, passing the $CHCl_3$ soln, when cleared, thru a small, dry filter into a 200 cc Erlenmeyer flask; and distil the $CHCl_3$ by the aid of gentle heat to about 20 cc. Add 10 cc of $H_2SO_4$ (1+9) and digest on a steam bath until the residue has been reduced ½; add 20 cc of $H_2O$ and continue the digestion for an hour; then add a second 20 cc portion of $H_2O$ and 10 cc of HCl; and titrate very slowly, dropwise, with the standard bromide-bromate soln, **25** (**c**), until a faint yellow color remains. While adding this reagent, rotate the flask sufficiently to agglomerate the precipitated tribromanilin. Calculate the quantity of acetanilid present.

If the preparation contains caffeine or antipyrin, or both, in addition to acetanilid and acetphenetidin, proceed as follows: (1) Digest the mixture by heating with the $H_2SO_4$ (1+9) to convert acetphenetidin and acetanilid to phenetidin and anilin sulfates, respectively; (2) separate the caffeine and antipyrin by extraction with $CHCl_3$; (3) regenerate acetphenetidin and acetanilid by treating the soln of the corresponding sulfates with solid $NaHCO_3$ in slight excess and adding a few drops of acetic anhydride, and extract with $CHCl_3$.[3]

## ACETANILID AND CAFFEINE[3]—TENTATIVE

### 5 REAGENTS

(**a**) *Standard bromide-bromate soln.*—Dissolve 15 g of $KBrO_3$ and 100 g of KBr in $H_2O$. Dilute to 1 liter and standardize against recrystallized and dried acetanilid, according to **4**(**b**), beginning with "Add 10 cc of $H_2SO_4$(1+9)."

(**b**) *Chloroform.*—Redistilled and residue-free.

(**c**) *Wagner's reagent.*—Dissolve 2 g of I and 6 g of KI in a minimum quantity of $H_2O$ and dilute to 100 cc.

Treat all corks used in the distillation with $CHCl_3$.

### 6 PREPARATION OF SOLUTION

(**a**) If the sample is already in powder, rub thoroly in a mortar and keep in a tightly corked tube or flask. Powders in paper, cachet, or capsule containers are frequently of such fineness as to require little further trituration except to produce a uniform product. With tablets and pills, determine their average weight and powder in a mortar. Weigh 0.3–0.5 g of the sample or, if preferred, a quantity equal to, or a multiple of, the average unit dose (previously ascertained by weighing collectively 20 or more such doses); transfer to a separator; add 50 cc of $CHCl_3$ and 20 cc of $H_2O$; shake vigorously; and, after clearing, draw off the lower layer thru a small, dry filter into a 200 cc Erlenmeyer flask. Repeat the extraction twice, using 50 cc portions of $CHCl_3$ for each extraction. Recover any caffeine-acetanilid mixture observable about the apex of the delivery tube of the separator, edge of filter, and tip of separator by careful washing with $CHCl_3$ and add these washings to the main portion. Distil the combined $CHCl_3$ extracts to about 10 cc.

If the caffeine is present, as the free alkaloid or in other readily extractable form, the extraction may, if preferred, be made on filter paper by washing with successive 5–10 cc portions of $CHCl_3$ (30–50 cc is usually sufficient) until the extraction is complete, as indicated by the absence of any residue after evaporation of a small portion of the last washing.

**(b)** In the case of dilute alcoholic solns, evaporate a measured quantity on a steam bath until most of the alcohol has been expelled, or take an aliquot of the residue from an alcohol determination and transfer to a separator by pouring and rinsing with a minimum quantity of $H_2O$ so that the final volume does not greatly exceed 20 cc. In order to avoid any loss of acetanilid by hydrolysis during evaporation, add a little solid $NaHCO_3$ and a drop of acetic anhydride. (Should the preparation contain alkaloids, acidify with a few drops of $H_2SO_4$ (1+9) immediately after acetylization to retain such basic material in the aqueous soln.) Add 50 cc of $CHCl_3$; shake vigorously; and, after clearing, draw off the $CHCl_3$ layer thru a filter into a 200 cc Erlenmeyer flask. Repeat the extraction twice, using 50 cc portions of $CHCl_3$ for each extraction, and distil the combined $CHCl_3$ washings to a volume of about 10 cc.

### 7 DETERMINATION

**(a)** *Caffeine.*—Treat the $CHCl_3$ soln obtained under **6** with 10 cc of $H_2SO_4$ (1+9) and digest on a steam bath until the contents of the flask are reduced to 5 cc. Add 10 cc of $H_2O$ and continue the digestion until the liquid is again reduced to 5 cc, cool, and transfer to a separator with a minimum of $H_2O$. (The final volume should not greatly exceed 20 cc.) Add 50 cc of $CHCl_3$; extract in the usual way; and, after clearing, withdraw the lower layer thru a small, dry filter into a 200 cc Erlenmeyer flask. Repeat the extraction with two 50 cc portions of $CHCl_3$. Distil the combined extracts to about 10 cc, finally transferring the residual liquid, by washing with $CHCl_3$, to a weighed beaker or crystallizing dish. Allow the soln to evaporate spontaneously, or by gentle heat and an air blast, to apparent dryness. Cool, and allow to stand in the open until weight becomes constant.

From those preparations that contain powdered cinnamon, celery seed, ginger, or other vegetable products, $CHCl_3$ extracts, in addition to caffeine and acetanilid, certain oils, fats, waxes, resins, pigments, and other substances. After the caffeine-acetanilid mixture has been digested, these oils, etc., appear either in suspension or soln and contaminate the caffeine. Remove any suspended impurities by filtering thru a small, moistened filter immediately after hydrolysis and prior to extraction with $CHCl_3$. Should the recovered caffeine be deeply colored or contaminated with foreign matter, purify it as follows: Dissolve in $H_2SO_4$ (about 5 cc of 0.2 *N* acid for every 100 mg of caffeine); filter, if necessary, thru a moistened filter; add 15–20 cc of Wagner's reagent, sufficient to color distinctly the supernatant liquid a deep claret; stir; and allow to stand an hour, preferably in a refrigerator. Filter and wash the periodide with a few cc of I soln; transfer both filter and precipitate to a separator, using not more than 20 cc of $H_2O$; decolorize with a crystal of $Na_2S_2O_3$; then extract with three 50 cc portions of $CHCl_3$; and proceed as directed above.

(b) *Acetanilid.*—Transfer the soln of anilin sulfate remaining in the separator to the Erlenmeyer flask used in effecting hydrolysis and heat 10 min. on a steam bath to expel all traces of $CHCl_3$. Wash with 5 cc of $H_2O$ the filter that was used in drying the $CHCl_3$ soln of caffeine, adding the washings to the main soln of anilin sulfate. Add 10 cc of HCl and titrate with the standard bromide-bromate soln until a faint yellow coloration remains, rotating the flask sufficiently to agglomerate the precipitated tribromanilin. Calculate the quantity of acetanilid from the number of cc required to complete the precipitation.

Caffeine and acetanilid mixtures frequently contain $NaHCO_3$ also, which appears as the $CHCl_3$-insoluble residue and may be determined by titrating such residue with standard acid, congo red indicator being used. The bicarbonate may also be determined by igniting the original sample, or the $CHCl_3$-insoluble residue, with $H_2SO_4$ and weighing the resulting $Na_2SO_4$.

Should the "acetanilid compound" be combined with NaBr, the bromide, in the absence of other halides, may be determined volumetrically as directed under **XII, 35**.

## ACETANILID, CAFFEINE, AND CODEINE—TENTATIVE

### 8 PREPARATION OF SOLUTION

Transfer to a separator one or more average unit doses (about 0.225 g of acetanilid) of the powdered sample; add 20 cc of $H_2O$, 50 cc of $CHCl_3$, and 10 drops of $H_2SO_4$ $(1+9)$; and extract in the usual way. After clearing, wash the solvent in a second separator with 5 cc of $H_2O$ and transfer to a 200 cc Erlenmeyer flask. Repeat the foregoing operations with two 50 cc portions of $CHCl_3$, finally distilling the combined $CHCl_3$ soln by gentle heat to about 10 cc.

### 9 DETERMINATION

(a) *Acetanilid and caffeine.*—Treat the $CHCl_3$ residue obtained under **8** as directed under **7**.

(b) *Codeine.*—Combine the wash $H_2O$ used in the second separator under **8** with the soln of codeine sulfate; add an excess of solid $NaHCO_3$; extract with 5 successive portions of 30, 25, 20, 15, and 10 cc of $CHCl_3$; wash the combined $CHCl_3$ extracts with 5 cc of $H_2O$ in a second separator; and pass thru a dry filter into a 200 cc Erlenmeyer flask. Distil by gentle heat to about 5 cc. Transfer the $CHCl_3$ soln to a small weighed beaker, evaporate to apparent dryness on a steam bath, add a few drops of alcohol and a like quantity of water to the amorphous residue, and evaporate again. Finally cool and allow the usually crystalline product to stand until the weight becomes constant. Check this result volumetrically by dissolving the residue in 3–5 cc of neutral alcohol and titrating with 0.02 $N$ $H_2SO_4$ to a faint red color, using methyl red indicator [**38**(**e**)].

The quantity of codeine as found by weight will usually be slightly greater than that determined by titration. In order to insure the greatest possible accuracy in the volumetric operations the strength of the standard acid used should be checked by titration against pure codeine.

## ACETANILID, CAFFEINE, AND QUININE—TENTATIVE

### 10 PREPARATION OF SOLUTION

Proceed as directed under **8**.

### 11 DETERMINATION

(a) *Acetanilid and caffeine.*—Proceed as directed under **7.**

(b) *Quinine.*—Combine the wash $H_2O$ used in the second separator under **8** with the soln of quinine bisulfate, add a slight excess of $NH_4OH$, and extract with three 50 cc portions of $CHCl_3$. Wash each portion with 5 cc of $H_2O$ in a second separator and pass thru a dry filter into a 200 cc Erlenmeyer flask. Distil by gentle heat to about 5 cc; evaporate on a steam bath to apparent dryness; dissolve the amorphous alkaloid in 5 cc of neutral alcohol; and titrate with 0.02 *N* acid to a faint red, using 2 drops of methyl red indicator [**38**(e)]. Heat on a steam bath until most of the alcohol has been expelled, adding, if necessary, sufficient acid to maintain the acid reaction. From the total number of cc of acid used in the titration calculate the quinine present. 1 cc of 0.02 *N* acid = 7.567 mg of quinine ($C_{20}H_{24}O_2N_2.3H_2O$) or 8.728 mg of quinine sulfate $[C_{20}H_{24}O_2N_2)_2\ H_2SO_4.7H_2O]$ or 8.908 mg of quinine-sulfate $(C_{20}H_{24}O_2N_2)_2,\ H_2SO_4.8H_2O$, or 7.936 mg of quinine hydrochloride ($C_{20}H_{24}O_2N_2HCl.2H_2O$) or 7.945 mg of quinine dihydrochloride ($C_{20}H_{24}O_2N_2.2HCl$).

If the mixture contains acetphenetidin in place of acetanilid, proceed as outlined above, except to conduct the separation of caffeine and acetphenetidin as directed under **17.**

## ACETANILID, CAFFEINE, QUININE, AND MORPHINE[4]—TENTATIVE

### 12 PREPARATION OF SOLUTION

Transfer to a separator a quantity (containing not less than ¼ grain of morphine) of the powdered sample equal to, or a multiple of, a unit dose and add 20 cc of $H_2O$ and 10 drops of $H_2SO_4$ (1+9). Extract with three 50 cc portions of alcohol-free $CHCl_3$, wash each portion in a second separator with 5 cc of $H_2O$, and add the combined washings to the alkaloidal soln in the first separator. Filter the $CHCl_3$ extracts through a small, dry filter into a 200 cc Erlenmeyer flask and distil by gentle heat to about 10 cc.

### 13 DETERMINATION

(a) *Acetanilid and caffeine.*—Proceed as directed under **7**, using the $CHCl_3$ extract obtained under **12.**

(b) *Quinine.*—Add to the soln of quinine and morphine sulfates, obtained under (a), 4–5 cc of a 10% NaOH soln and extract with four 40 cc portions of $CHCl_3$. Wash each portion with 5 cc of $H_2O$ and pass the clear solvent thru a small, dry filter into a 200 cc Erlenmeyer flask. Remove the solvent by gentle distillation and titrate the residual quinine with 0.02 *N* acid as directed under **11**(b). (If the morphine salt present is contaminated with codeine, the latter will be separated and titrated with the quinine.)

(c) *Morphine.*—Wash the filter used under (b) with 5 cc of $H_2O$ and add the washings to the aqueous, alkaline soln of the alkaloid. Add 0.5 g of $NH_4Cl$ (or a quantity slightly in excess of that required to free the morphine as well as convert all NaOH to NaCl), 45 cc of $CHCl_3$, and 5 cc of alcohol. Extract in the usual way, washing the solvent in a second separator with 5 cc of $H_2O$. After clearing, pass the $CHCl_3$ thru a small, dry filter into a 200 cc Erlenmeyer flask. Repeat the extraction with three 40 cc portions of $CHCl_3$, washing and filtering as before. Collect all the solvent in an Erlenmeyer flask and distil to about 10 cc. Transfer with $CHCl_3$ to a small beaker and evaporate to apparent dryness. Dissolve the residue in 1–2 cc of warm, neutral methyl alcohol; add a drop of methyl red indicator [**38**(e)]; and titrate with 0.02 *N* acid to a faint red color. Evaporate most of the alcohol on a steam bath and, if necessary, add from a buret sufficient of the 0.02 *N* acid to maintain the faint red color. From the total volume of 0.02 *N* acid used calculate the morphine present. In order to insure the greatest possible accuracy, the strength of the standard acid used should be checked by titration against pure morphine.

In the various operations involving fixation and subsequent liberation of morphine by means of fixed alkali and $NH_4Cl$, the most careful attention should be paid to the manner of adding the reagents, since any undue excess of either might nullify the entire procedure. Any large excess of NaOH would naturally require for its reduction a correspondingly large quantity of $NH_4Cl$, the latter in turn yielding its equivalent of hydroxide, relatively large quantities of which, thru interaction with NaCl, tend to inhibit any permanent liberation of alkaloid and thus prevent complete extraction. Furthermore, $NH_4Cl$ in large quantity operates retentively on the morphine in soln, due in part possibly to the formation of an alkaloidal hydrochloride.

### ACETANILID AND SODIUM SALICYLATE[5]—TENTATIVE

### 14 PREPARATION OF SOLUTION

Weigh a quantity of the powdered sample equal to, or a multiple of, an average unit dose (about 0.225 g of acetanilid); transfer to a separator containing 10 cc of $H_2O$; and, for every unit dose, add 0.1 g of solid $NaHCO_3$. In the case of tablets and pills, ascertain their average weight; powder in a mortar; and weigh a quantity of the powder equivalent to one or more tablets or pills for each determination. In the examination of alcoholic preparations, distil the alcohol from a measured volume on a steam bath, transfer to a separator with a minimum quantity of $H_2O$, and add 0.5–1.0 g of solid $NaHCO_3$.

### 15 DETERMINATION

(a) *Acetanilid.*—Extract the alkaline soln obtained under **14** with three 50 cc portions of $CHCl_3$; wash each portion with 5 cc of $H_2O$ in a second separator and collect the solvent, without previous drying, in a 200 cc Erlenmeyer flask. Reserve the aqueous soln for the determination of Na-salicylate under (b). Distil the $CHCl_3$ very gently to about 5 cc, add 10 cc of $H_2SO_4$ (1+9), and completely hydrolyze on a steam bath. Proceed from this point as directed under **7**(b), beginning with "Add 10 cc of HCl."

(b) *Sodium salicylate.*—Acidify the aqueous soln of Na-salicylate, under (a), with a few drops of HCl and extract 3 to 5 times with 25 cc portions of $CHCl_3$ to exhaust the salicyclic acid present in the mixture. Treat each portion in a second separator with 20 cc of $H_2O$ containing 1 g of anhydrous $Na_2CO_3$ for very 100 mg of salicyclic acid. Shake vigorously and, after clearing, wash each portion again in a third separator with 5 cc of $H_2O$. Add the washings to the main aqueous alkaline soln of Na-salicylate. Dilute to a known volume; transfer an aliquot, representing about 100 mg of salicyclic acid, to a 200 cc Erlenmeyer flask; dilute to 60–75 cc; heat nearly to boiling; add slowly 50–80 cc of approximately 0.1 *N* I soln, sufficient to insure an excess during digestion; and digest for an hour on a steam bath. Remove the free I with a few drops of $Na_2S_2O_3$ soln and decant the clear liquid thru a weighed Gooch crucible, retaining most of the precipitate, tetraiodophenylenequinone $(C_6H_2I_2O)_2$ in the flask. To the latter add 50 cc of boiling $H_2O$; digest 10 min. on a steam bath; filter; and wash gradually all the precipitate into the Gooch crucible, using for this purpose and the final washing about 200 cc of hot $H_2O$. Dry to constant weight in an air bath at 100°. Multiply the weight of the precipitate by 0.4654 to obtain the quantity of Na-salicylate present in the aliquot taken.

Should the mixture contain caffeine or antipyrin, or both, these substances will appear with the acetanilid in the first $CHCl_3$ extract and may be determined as directed in the remarks following **32(b)**. Should the acetanilid be replaced by acetphenetidin in the mixture, the general procedure would not be materially altered, the acetphenetidin being weighed directly after recovery from its washed $CHCl_3$ soln as separated from the Na-salicylate. If, instead of Na-salicylate, the mixture contains the free acid or its $NH_4$ salt, add a larger quantity of $NaHCO_3$ prior to extraction with $CHCl_3$ to insure the fixation of salicyclic acid.

In the analysis of a mixture of caffeine, acetanilid, Na-salicylate, and codeine, the following procedure is recommended: (1) Extraction of caffeine, acetanilid and salicylic acid from the acidified soln; (2) washing the $CHCl_3$ soln with aqueous $Na_2CO_3$ soln for the recovery of the salicylic acid, preliminary to its treatment with I soln; (3) separation of caffeine and acetanilid as directed under **7(a)** and **7(b)**; and (4) recovery of codeine from the soln of its sulfate after treatment with $NaHCO_3$ and $CHCl_3$.

## ACETPHENETIDIN (PHENACETIN) AND CAFFEINE[6]—TENTATIVE

### 16 PREPARATION OF SOLUTION

In the case of preparations containing acetphenetidin instead of acetanilid, but otherwise identical, make the gross separation of the caffeine-acetphenetidin mixture as directed under **6**.

### 17 DETERMINATION

**(a)** *Caffeine.*—Treat the $CHCl_3$ extract obtained under **16** with 10 cc of $H_2SO_4$ (1+9) and digest on a steam bath until the liquid is reduced to about 5 cc. Dilute with 10 cc of $H_2O$ and continue the digestion until the volume is again reduced to 5 cc; again add 10 cc of $H_2O$ and continue heating until the residual liquid amounts to 8–10 cc. If, during the digestion, particles of acetphenetidin remain on the sides of the flask, rinse them into the soln with a few drops of $CHCl_3$. (Great care must also be given to the degree of evaporation. Should the aqueous-acid soln and suspension of caffeine-acetphenetidin be concentrated much beyond the limits indicated, more or less phenetidin sulfonate is likely to be formed, which later resists acetylization and conversion to acetphenetidin.) Cool, transfer with water to a separator so that the final volume does not greatly exceed 20 cc, and proceed as directed under **7(a)**.

**(b)** *Acetphenetidin.*—Wash the filter used to dry the $CHCl_3$ with 5 cc of $H_2O$, receiving the washings in the separator containing the soln of phenetidin sulfate. Treat with successive small portions of solid $NaHCO_3$ until, after complete neutralization of free acid, an excess of $NaHCO_3$ remains. Add 50 cc of $CHCl_3$ and, for every 0.1 g of acetphenetidin known or believed to have been present, 5 drops of acetic anhydride; shake vigorously; allow to clear; and withdraw the $CHCl_3$ into a second separator containing 5 cc of $H_2O$. Shake this mixture and, after clearing, pass the solvent thru a small, dry filter into a 250 cc Erlenmeyer flask. Repeat the extraction twice with 50 cc portions of $CHCl_3$, washing each portion with the 5 cc of $H_2O$ in the second separator. Distil the combined $CHCl_3$ extractions to about 10 cc, transfer the residual soln with sufficient fresh solvent to a weighed 50 cc beaker or crystallizing dish, evaporate on a steam bath to apparent dryness, and finally remove any considerable excess of acetic anhydride by repeated additions and evaporations of 1 cc of $CHCl_3$ and a drop of alcohol. (The reformed acetphenetidin should finally appear as a whitish, crystalline mass with a faint, acetous odor that disappears completely on standing some hours in the open or over lime in a vacuum desiccator.)

Weigh at intervals until the final weight differs from the preceding by not more than 0.5 mg.

### ACETPHENETIDIN (PHENACETIN) AND SALOL[7]

### 18 *Acid Hydrolysis Method—Tentative*

(**a**) *Acetphenetidin.*—Weigh on a tared 5.5. cm filter a quantity of the sample equal to, or a multiple of, the average weight of a unit dose and wash with sufficient successive, small portions of $CHCl_3$ to extract completely all acetphenetidin and salol present in the mixture (about 40 cc). Collect the soln in a weighed, 100 cc beaker and evaporate on a warm plate (50–60°) to apparent dryness, using an air blast. Let stand 24 hours at room temp. to practically constant weight and weigh. Transfer the crystalline residue, by means of $CHCl_3$ to a 50 cc lipped Erlenmeyer flask; evaporate the solvent by means of an air blast and gentle heat; add 10 cc of $H_2SO_4$ (1+9); and evaporate on a steam bath until the volume is reduced one-half. Add 10 cc of $H_2O$ and continue the digestion as before. Add a second 10 cc of $H_2O$ and evaporate to 5 cc. Transfer the residue with about 20 cc of $H_2O$ to a small separator and extract the salol with 15, 10, and 5 cc of $CHCl_3$, washing each extract with 5 cc of $H_2O$ in a second separator to recover traces of phenetidin sulfate possibly dissolved by the $CHCl_3$. Add the wash water in the second separator to the solution of phenetidin sulfate in the first separator and proceed as directed under **17**(**b**), beginning with "Treat with successive small portions of solid $NaHCO_3$."

(**b**) *Salol.*—Subtract the weight of acetphenetidin from the combined weight of the two ingredients to obtain the weight of salol.

### 19 *Alkaline Hydrolysis Method—Tentative*

(**a**) *Acetphenetidin.*—On a small, tared filter weigh a quantity of the sample containing not more than 0.08 g of salol; exhaust with $CHCl_3$ as directed under **18**(**a**); collect the solvent in a small, lipped Erlenmeyer flask; and evaporate the $CHCl_3$ by means of an air blast without heat. Add 10 cc of 2.5% NaOH soln and heat 5 min. on a steam bath. Cool quickly to room temp. in running $H_2O$ to prevent partial hydrolysis of the acetphenetidin. Transfer the liquid to a separator with a minimum quantity of $H_2O$ and rinse the flask with the first 20 cc portion of $CHCl_3$ to be used in the following extraction. Extract the alkaline soln with three 20 cc portions of $CHCl_3$; wash each portion in a second separator with 5 cc of $H_2O$; and pass the $CHCl_3$ soln thru a small, dry filter into a 200 cc Erlenmeyer flask. Reserve the combined alkaline soln and washings for the determination of salol under (**b**). Distil the combined $CHCl_3$ extracts to about 5 cc. Transfer by means of a little $CHCl_3$ to a small, weighed beaker or crystallizing dish; evaporate on a steam bath with the aid of an air blast; cool; and weigh the residual acetphenetidin at intervals until the weight becomes constant.

(**b**) *Salol.*—Place the reserved combined alkaline soln and washings under (**a**) in a 500 cc glass-stoppered bottle; dilute with $H_2O$ to about 200 cc; run in from a buret an excess (about 50 cc) of 0.1 *N* bromide-bromate soln; add 10 cc of HCl; and shake 1 min., then at intervals for 30 min. Add 10 cc of 15% KI soln and shake at intervals for 15 min. Titrate the free I with standard $Na_2S_2O_3$ soln previously standardized against the 0.1 *N* bromide-bromate soln. 1 cc of 0.1 *N* bromide-bromate soln = 1.784 mg of salol.

### ACETYLSALICYLIC ACID[8]

### 20 *Melting Point—Official, First Action*

If excipients are present, treat 0.2–0.3 g with small portions of $CHCl_3$ and filter into a beaker or evaporating dish. Evaporate the bulk of the $CHCl_3$ on a steam bath and complete by spontaneous evaporation until thoroly dry. Determine the melting point of the crystalline residue as directed under **XXI, 56.**

### FREE SALICYLIC ACID

**21** *Qualitative Test—Official*

Shake a 0.5 g sample in a small Erlenmeyer flask with about 10 cc of $CHCl_3$ and filter. Evaporate, treat the residue with 10 cc of cold $H_2O$, and filter. Add 1 drop of a 10% $FeCl_3$ soln. Only a very faint violet color should result.

*Quantitative Method—Official*

**22** REAGENTS

(a) *Standard salicylic acid soln.*—Dissolve 0.1 g of pure salicylic acid in 5 cc of alcohol and dilute with $H_2O$ to 1 liter.

(b) *Ferric ammonium sulfate soln.*—Dissolve 1 g [$FeNH_4(SO_4)_2 . 12H_2O$] in 100 cc of $H_2O$.

**23** DETERMINATION

In a colorimetric tube dissolve 0.2 g of the sample in 1 cc of alcohol and add 48 cc of $H_2O$. Filter, if necessary. In a second colorimetric tube place 5 cc of the standard salicylic acid soln, 1 cc of alcohol, and 43 cc of $H_2O$. Add 1 cc of the $FeNH_4(SO_4)_2$ soln to each tube. Mix, let stand 5 min., and compare the colors in a colorimeter.

### TOTAL SALICYLATES

**24** *Iodine Method*[9]*—Official*

Weigh a 0.1 g sample into a 200 cc Erlenmeyer flask, add 20 cc of $H_2O$ and 1 g of $Na_2CO_3$, and heat on a steam bath for 15 min. Filter, if necessary, to remove talc. Dilute to 60–75 cc, heat nearly to boiling, add slowly an excess (50–80 cc) of approximately 0.1 *N* I soln, and proceed as directed under **15**(b) (sodium salicylate). Multiply the weight of the precipitate by 0.4015 to obtain the total salicylic acid and deduct the free salicylic acid as determined under **23.** Multiply the remainder by 1.304 to obtain the weight of acetylsalicylic acid.

*Bromine Method—Official*

**25** REAGENTS

(a) *Sodium hydroxide soln.*—Dissolve 2 g of NaOH in $H_2O$ and dilute to 100 cc.

(b) *Potassium iodide soln.*—Dissolve 20 g of KI in $H_2O$ and dilute to 100 cc.

(c) *Standard bromide-bromate soln* (0.1 *N bromine soln.*)*—Dissolve 3 g of $KBrO_3$ and 50 g of KBr in $H_2O$ and dilute to 1 liter. Standardize 30 cc of the Br soln by transferring to a glass-stoppered Erlenmeyer flask and adding 25 cc of $H_2O$, 5 cc of KI soln, and 5 cc of HCl. Shake thoroly. Titrate with 0.1 *N* $Na_2S_2O_3$ soln, using starch indicator [**VI, 3**(e)].

**26** DETERMINATION

Saponify a 0.5 g sample with 10 cc of the NaOH soln by heating for 15 min. on a steam bath. Dilute with $H_2O$ in a volumetric flask to 500 cc. Transfer an aliquot of this soln, representing not less than 0.04 g nor more than 0.05 g of acetylsalicylic acid, to a 500 cc glass-stoppered Erlenmeyer flask; add 30 cc of the standard bromide-bromate soln and 5 cc of HCl; and immediately insert the stopper. Shake repeatedly for 30 min. and allow to stand for 15 min. Remove the stopper just sufficiently to introduce quickly 5 cc of the KI soln, taking care that no Br vapors escape, and immediately stopper the flask. Shake thoroly, remove the stopper, and rinse it and the neck of the flask with a little $H_2O$ so that the washings flow into the flask. Titrate with 0.1 *N* $Na_2S_2O_3$ soln, using starch indicator [**VI**, **3**(e)]. 1 cc of 0.1 *N* bromine-bromate soln = 2.30 mg of salicylic acid, or 3 mg of acetylsalicylic acid.

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* See note 7, page xvii.

### *Double Titration Method for Acetylsalicylic Acid*[10]*—Official*

### 27 PREPARATION OF SOLUTION

(**a**) *Dry extraction method* (*applicable in all cases*).—Treat a quantity of sample containing not less than 0.3 g of acetylsalicylic acid, accurately weighed, with small portions of $CHCl_3$, filter into a beaker, and wash with $CHCl_3$ until completely extracted. Evaporate the bulk of the $CHCl_3$ on a steam bath, finishing with the aid of an electric fan without heat.

(**b**) *Wet extraction method* (*applicable in the absence of acids and alkaline or alkali earth carbonates*).—Transfer the accurately weighed sample to a small separator containing about 20 cc of $H_2O$. Shake out repeatedly with $CHCl_3$, using successively 30, 25, 20, 15, 10, and 5 cc portions, and test for completeness of extraction by evaporating on a watch-glass a portion of the final extraction. Filter the combined $CHCl_3$ portions through cotton and wash the funnel and cotton with $CHCl_3$. Evaporate the bulk of the $CHCl_3$ on a steam bath, finishing with the aid of an electric fan without heat.

(**c**) *Acetylsalicylic acid and uncoated tablets containing no excipient.*—Dissolve the sample directly in 10 cc of alcohol.

### 28 DETERMINATION

Dissolve the dry $CHCl_3$ extract in 10 cc of neutral alcohol and titrate immediately with 0.1 *N* alkali soln, using phenolphthalein indicator. Make this titration rapidly and use the first persistent pink color as the end point, since any slight excess of alkali has a tendency to hydrolize the ester quickly. Add a volume of the 0.1 *N* alkali equal to that used in the first titration and then add 5 cc more. Heat on a steam bath for 15 min. Titrate back with 0.1 *N* acid. If the product is pure, the total quantity of alkali consumed will be twice that of the first titration. Each cc of 0.1 *N* alkali consumed in the 2 titrations is equivalent to 9 mg of acetylsalicylic acid.

### 29 COMBINED ACETIC ACID IN ACETYLSALICYLIC ACID[11]—OFFICIAL

Weigh accurately 2 g of the powdered material and transfer to a separator, using about 25 cc of $H_2O$. Extract completely with $CHCl_3$, testing the last extraction by evaporating a small quantity of the $CHCl_3$ to dryness. (Usually 6 extractions with 30, 25, 20, 10, 10, and 5 cc portions of $CHCl_3$ are sufficient.) Collect the $CHCl_3$ fractions in a beaker and filter thru a pledget of cotton into a weighed beaker that has been counterpoised previously with a beaker of the same dimensions, similarly dried, and exposed to the air. Wash the original beaker, funnel, and cotton with $CHCl_3$ and add these washings to the $CHCl_3$ soln in the weighed beaker. Evaporate the $CHCl_3$ on a steam bath, dry the residue at 80° for 15 min., and weigh, using the counterpoised beaker similarly treated. From the weight calculate the $CHCl_3$ extract.

Treat the $CHCl_3$ extract, or if no excipients are present, 2 g of the powdered material, in a 150 cc beaker with 30 cc of *N* NaOH and evaporate on a steam bath nearly to dryness. Transfer to a separator, using for this operation 10 cc of $H_2O$, 20 cc of $H_2SO_4$ (6+94), and finally two 5 cc portions of $H_2O$. Extract with successive portions of $CHCl_3$, using the first fraction of 50 cc to rinse the beaker in which the saponification was carried on. Continue the extractions with $CHCl_3$ until all salicylic acid is removed. (This generally requires about 6 extractions.) During these extractions keep the stopper in the separator to guard against loss of acetic acid by evaporation. Collect the $CHCl_3$ fractions in a second separator and wash with 25 cc of $H_2O$ and wash this $H_2O$ once with 5 cc of $CHCl_3$. Discard the $CHCl_3$ extractions and return the wash $H_2O$ to the acid $H_2O$ in the first separator. Transfer the acid $H_2O$ containing acetic and $H_2SO_4$ to a 200 cc volumetric flask, wash the separators thoroly with $H_2O$, add to the flask, dilute to volume, and mix thoroly. Pipet two 50 cc portions, using the same pipet and draining it the same length of time. Place one portion in a receptacle suitable for titration and the other in a large Pt dish. Titrate the first portion at once with 0.5 *N* alkali, using phenolphthalein indicator. Evaporate the portion in the Pt dish on a steam bath to dryness, take up in 10 cc of $H_2O$, and again evaporate, repeating this process twice more. (During evaporation guard against contact with $NH_3$ vapors.) Take up the residue in $H_2O$ and titrate with 0.5 *N* alkali, using phenolphthalein indicator. Subtract the second titration reading from the first and calculate the percentage of acetic acid on a 0.5 g sample. 1 cc of 0.5 *N* alkali = 0.03002 g of acetic acid.

### 30 ACETYLSALICYLIC ACID IN MIXTURES CONTAINING ACETPHENETIDIN AND CAFFEINE[12]—OFFICIAL

Ascertain the average weight of a number of tablets and reduce to a fine powder.

Weigh accurately 0.2 g of the powder, transfer to a separator with about 25 cc of $H_2O$, and extract carefully with repeated portions of $CHCl_3$. Test the final extraction by evaporating a small portion on a steam bath to dryness. (No residue should remain if the extraction is complete. About 6 extractions are generally required, and these can be made with 30, 25, 20, 10, 10, and 5 cc portions of $CHCl_3$.) Collect the $CHCl_3$ fractions in a separator and draw off into a 200 cc Erlenmeyer flask, placing a pledget of cotton in the stem of the separator to filter the $CHCl_3$. Wash the separator twice with 5 cc portions of $CHCl_3$, passing this through the cotton and leaving any $H_2O$ that may have separated in the separator. Add the $CHCl_3$ washings to the flask and evaporate the $CHCl_3$ on a steam bath to a volume of about 2 cc. Add 10 cc of $H_2SO_4$ (1+9), connect with a reflux condenser, and digest for 30 min., partially immersing the flask in a boiling water bath. Cool, and transfer to a separator, rinsing the condenser with $CHCl_3$ and using a minimum quantity of $H_2O$ to effect the transfer, so that the final volume does not greatly exceed 20 cc. Extract the caffeine and salicylic acid with 6 portions of $CHCl_3$, using 30, 25, 20, 15, 10, and 10 cc for the extractions. Collect these fractions in a separator, add 20 cc of $H_2O$ and 1 g of $Na_2CO_3$, and shake thoroly. Drain off the $CHCl_3$ into another separator and wash twice more with 15 and 10 cc of $H_2O$. Reject the $CHCl_3$ and combine the $Na_2CO_3$ soln and wash waters in a 200 cc Erlenmeyer flask. Heat on a steam bath to expel traces of $CHCl_3$, dilute to 100 cc with $H_2O$, then add slowly 25–40 cc of strong I soln (about 0.2 *N*), sufficient to insure excess during digestion, and digest 1 hour on the steam bath. Remove the free I with a few drops of $Na_2S_2O_3$ soln. Decant the clear soln thru a weighed Gooch, retaining most of the precipitate in the flask. To the latter add 50 cc of boiling $H_2O$, digest 10 min. on the steam bath; filter; and wash gradually all the precipitate into the Gooch, using altogether about 200 cc of hot $H_2O$ to complete the operation. Dry to constant weight in an air bath at 100° and weigh the precipitate of tetraiodophenylenequinone $(C_6H_2I_2O)_2$. The weight of precipitate multiplied by 0.4016 gives the total salicylic acid present. If free salicylic acid is present, this should be deducted from the total, and the difference times 1.304 is the weight of acetylsalicylic acid.

## ANTIPYRIN AND CAFFEINE[13]—TENTATIVE

### 31 PREPARATION OF SOLUTION

(a) Weigh a quantity of the finely powdered sample equal to, or a multiple of, an average unit dose; transfer to a filter; and extract with $CHCl_3$ to separate the caffeine and antipyrin from the usual excipients of tablet and pill combinations. Distil the greater part of the $CHCl_3$ and evaporate the remainder on a steam bath.

(b) With alcoholic preparations, remove the alcohol from a measured quantity of the sample by heating on a steam bath. Extract the residue with three 50 cc portions of $CHCl_3$ in a separator. Distil the greater portion of the $CHCl_3$ and evaporate the remainder on a steam bath.

### 32 DETERMINATION

(a) *Antipyrin.*—Transfer the residue obtained under **31**, which should weigh about 0.25 g, to a 150 cc separator by means of two 5 cc portions of alcohol-free $CHCl_3$, followed by 10 cc of $H_2O$. Add 1 g of $NaHCO_3$ and 10–15 cc of 0.2 *N* I (or double the quantity of 0.1 *N* I), adding the latter in small portions and shaking the mixture vigorously after each addition. (The I should then be in excess of that required to convert all the antipyrin into the mono-iod derivative. If it is not, add a little more I and shake the mixture again.) Remove the free I with a small crystal of $Na_2S_2O_3$ and add 15 cc of washed $CHCl_3$, shaking vigorously for 1 min. After clearing, draw off the $CHCl_3$ soln into a second separator; wash with 5 cc of $H_2O$; filter thru a small, dry filter into a weighed 50 cc beaker; and evaporate to apparent dryness on a steam bath, using an air blast. Repeat the extraction with 2 (3, if 0.1 *N* I has been used) 25 cc portions of washed $CHCl_3$, wash, filter, and evaporate each portion as above. Recover any crystalline product separating about the tip of the delivery tube, funnel, and edge of filter by judicious washing with $CHCl_3$. Dry the nearly colorless, crystalline residue of caffeine and iodantipyrin 30 min. at 105°, cool, and weigh. Designate this weight as "*A*."

The use of alcohol-free $CHCl_3$ in connection with the halogenation of antipyrin is necessary in order to preclude the formation of $CHI_3$, the presence of which in the composite residue *A* would vitiate the result.

Dissolve the composite residue in 5 cc of glacial acetic acid, add 10 cc of saturated $SO_2$ soln, and transfer with hot water to a 400–500 cc beaker until the final volume amounts to about 200 cc. Add sufficient $AgNO_3$ soln to precipitate all the I (about 0.3 g of $AgNO_3$) and a few drops of $HNO_3$, heat nearly to boiling, and stir to agglomerate the AgI. Add 15 cc of $HNO_3$, cover the beaker with a watch-glass, and boil gently for 5 min. Filter by decantation through a weighed Gooch crucible; wash the precipitate once with a little alcohol, then with two 100 cc portions of boiling $H_2O$; and finally transfer the silver iodide to the crucible. Wash several times with hot $H_2O$ and again with alcohol to remove traces of organic matter, dry 30 min. in an air bath at 110°, cool, and weigh. The weight of AgI multiplied by 0.8014 gives the weight of antipyrin.

(b) *Caffeine.*—Calculate the quantity of caffeine by multiplying the weight of AgI by 1.3374 and subtracting the product from the weight *A*, under (a).

In the analysis of a mixture containing caffeine, antipyrin, acetanilid, and Na-salicylate, the following steps are essential in effecting a separation: (1) Extraction of caffeine, acetanilid, and antipyrin from the aqueous, alkaline soln with $CHCl_3$; (2) hydrolytic treatment with $H_2SO_4$ of the three substances thus separated preliminary to the determination of caffeine and antipyrin as directed under **32**.

## MICROCHEMICAL TESTS FOR ALKALOIDS[14]—OFFICIAL, FIRST ACTION

**33** REAGENTS

(a) *Disodium phosphate soln.*—Dissolve 5 g of $Na_2HPO_4.12H_2O$ in 100 cc of $H_2O$.

(b) *Kraut's reagent.*—Dissolve 8 g of $Bi(NO_3)_3.5H_2O$ in 20 cc of $HNO_3$, sp. gr. 1.8. Dissolve 27.2 g of KI in 50 cc of $H_2O$. Mix the solns and dilute to 100 cc.

(c) *Marme's reagent.*—Dissolve 3 g of $CdI_2$ in 18 cc of $H_2O$ containing in soln 6 g of KI.

(d) *Mercuric chloride soln.*—Dissolve 5 g of $HgCl_2$ in 100 cc of $H_2O$.

(e) *Millon's reagent.*—Dissolve metallic Hg in an equal weight of $HNO_3$ and dilute with an equal volume of $H_2O$.

(f) *Platinic chloride soln.*—Dissolve 5 g of $H_2PtCl_6$ in 100 cc of $H_2O$.

(g) *Potassium iodide soln.*—Dissolve 5 g of KI in 100 cc of $H_2O$.

(h) *Sodium benzoate soln.*—Dissolve 5 g of Na-benzoate in 100 cc of $H_2O$.

(i) *Sodium carbonate soln.*—Dissolve 5 g of $Na_2CO_3.H_2O$ in 100 cc of $H_2O$.

(j) *Wagner's reagent.*—Dissolve 1 g of I and 5 g of KI in 5 cc of $H_2O$ and dilute to 100 cc.

**34** PREPARATION OF SAMPLES

(a) *Controls.*—Dissolve 1 mg of the pure alkaloidal salt in 2 drops of $H_2O$ to make an approximately 1–100 soln.

(b) *Alkaloids in compounds.*—Separate the alkaloid in pure form by extracting it from ammoniacal soln with a suitable immiscible solvent, and evaporate the solvent. To 1 mg of the residue add, dropwise, 0.1 *N* HCl, avoiding an excess of acid, and dilute with water, if necessary to approximately the same alkaloidal concentration as used in (a).

(c) *Hypodermic tablets.*—Dissolve a portion of a tablet in $H_2O$ and dilute with $H_2O$ to approximately the same alkaloidal concentration as used in (a).

**35** IDENTIFICATION

Place a drop of the alkaloidal soln on a clean, glass slide; add a drop of reagent by means of a clean, glass rod; and, without stirring or covering, examine under the microscope, using low power. A magnification of 100–150 is suitable. Note the kind of crystals formed and compare their characteristics with the descriptions given and also with a control. (See table p. 451.)

**37** PILOCARPINE[15]—TENTATIVE

Ascertain the average weight per tablet. Pulverize, mix thoroly, and weigh out a sufficient portion to represent 1 grain of the salt. Dissolve the sample in 10 cc of $H_2O$, add 1 cc of dilute $NH_4OH$, and shake out rapidly with 20 cc of $CHCl_3$. Repeat the extraction, using 15 cc of $CHCl_3$, and complete with successive 10 cc portions. Filter each portion of the $CHCl_3$ drawn off through a pledget of cotton and combine in a 250 cc beaker, finally washing the stem of the separator and funnel with $CHCl_3$. Evaporate on the steam bath until the chloroformic soln measures about 5 cc. Add 20 cc of 0.02 *N* $H_2SO_4$ and evaporate the remainder of the $CHCl_3$. Titrate the excess acid with 0.02 *N* NaOH, using 1 drop of methyl red as indicator. (The end point is not particularly sharp, but with care it can be obtained.) 1 cc of 0.02 *N* $H_2SO_4 =$ 0.004893 g of $C_{11}H_{16}O_2N_2.HCl$.

## EMETINE HYDROCHLORIDE IN TABLETS[16]—TENTATIVE

**38** REAGENTS

(a) *Sodium hydroxide soln.*—4 g to 100 cc of $H_2O$.

(b) *Washed ether.*—Shake equal volumes of ether and $H_2O$ in a separator. Discard the $H_2O$.

*Characteristics of Microchemical Tests for Alkaloids*

| ALKALOID | REAGENT | DESCRIPTION OF CRYSTALS |
|---|---|---|
| Atropine | Wagner's | Small dark rods and triangular plates form in great numbers, singly and in groups |
| Brucine | Potassium iodide | Long masses of transparent, rectangular plates; also rosettes of thin plates |
| | Mercuric chloride | Small, dense rosettes |
| Caffeine | Mercuric chloride | Clusters of long, radiating, needle-shaped crystals |
| Cinchonidine | Sodium benzoate | Rosettes and sheaves of needles spreading to large size |
| | Platinic chloride | Rosettes of transparent plates |
| | Sodium carbonate | Spherical crystals, but not needles as in cinchonine |
| Cinchonine | Sodium carbonate | Dark rosettes composed of radiating needles form immediately |
| | Sodium phosphate | Similar to crystals formed by sodium carbonate, but more burr-shaped |
| Cocaine | Platinic chloride | Delicate, feathery crystals, later becoming heavier in structure |
| Codeine | Marme's | Silvery circular masses, crystallizing into dark rosettes of irregular outline |
| | Wagner's | Heavy, red-brown precipitate, crystallizing very slowly in yellow blades extending in branches (never red) |
| Ephedrine | Kraut's | Long, brown radiating and interlacing needles |
| Heroin[1] | Platinic chloride | Spherical clusters of golden yellow needles form slowly around a nucleus. |
| Morphine[2] | Marme's | Silvery, gelatinous precipitate, crystallizing in dense masses of fine needles |
| | Wagner's | Small drop of reagent produces heavy, red-brown precipitate, slowly crystallizing in shining, red, overlapping plates extending in branches |
| Pilocarpine | Platinic chloride | Crystals form slowly; layers of thin, yellow, triangular plates of delicate structure |
| Quinidine | Potassium iodide | Small, triangular crystals in great numbers, best in 1–1000 dilution; crystals soluble when excess reagent is added |
| Quinine | Disodium phosphate | Silvery, sheaflike crystals |
| Strychnine | Platinic chloride | Crystals form immediately in clusters and singly in small, wedge-shaped needles, which move about the field |
| | Marme's | Silvery masses, slowly forming rosettes |

[1] Official, see 76 (b).

[2] Official, see 68

(c) *Neutral alcohol.*

(d) *Sulfuric acid.*—0.02 *N*.

(e) *Methyl red indicator.*—Dissolve 0.2 g of methyl red in 100 cc of alcohol.

### PREPARATION OF SAMPLE

Weigh collectively all unbroken tablets and calculate the average weight per tablet. Powder a representative number of tablets, mix thoroly, and place in a weighing bottle.

### DETERMINATION

Transfer to a small separator sufficient of the powdered material, accurately weighed, to represent approximately 100 mg of the alkaloidal salt. Dissolve in a minimum of $H_2O$ and add 5 cc of the dilute NaOH soln. Extract with 30 cc of washed ether, draw off the aqueous soln, and swirl the separator to remove the $H_2O$ from the sides. Wash the ether with 1 cc of $H_2O$, adding the wash $H_2O$ to the aqueous soln. Decant the ether into a third separator, washing the mouth of the separator with ether. Repeat the extractions with 25, 20, 15 and 10 cc portions of ether or until extraction is complete, washing with 1 cc of $H_2O$ each time, and combine the ether extracts in the third separator. Filter into a beaker through cotton previously wet with ether, finally wash the separator with ether, and evaporate on the steam bath, using a low temp. to complete the evaporation.

To the residue add 2 cc of neutral alcohol, cover the beaker with a watch-glass, and allow to reflux on the steam bath for a few min. Add a few drops of methyl red, and without dilution titrate with 0.02 *N* acid to a faint pink. Cover the beaker and digest on a steam bath until all particles are completely dissolved. Cool, and add about 30 cc of recently boiled distilled $H_2O$. Finish the titration with standard acid to a faint red. 1 cc of 0.02 *N* $H_2SO_4 = 5.6946$ mg of emetine hydrochloride ($C_{15}H_{22}O_2N.HCl$).

### 39 ATROPINE IN TABLETS[17]—TENTATIVE

Weigh 25–100 tablets and introduce directly into a small separator. Dissolve in 5–20 cc of $H_2O$ and add 1 cc of $NH_4OH$. Add an equal volume of $CHCl_3$, agitate, and allow to stand until separation is complete. Draw off the $CHCl_3$ layer into a second separator and repeat the extraction with fresh portions of the solvent until the alkaloid is completely removed. After combining all the fractions, wash the combined $CHCl_3$ solns by agitation with 5 cc of $H_2O$ and allow to stand 15 min. Introduce a pledget of absorbent cotton into the stem of the separator and carefully draw off the $CHCl_3$ soln into a small beaker, but do not allow the wash $H_2O$ to enter the orifice of the stopcock. Add 10 cc of $CHCl_3$, agitate, and when the $H_2O$ has entirely risen to the surface draw off the $CHCl_3$ into the beaker. Wash the outer surface of the stem of the separator with a little $CHCl_3$, adding the washings to the beaker. Evaporate the soln on the steam bath to about 5 cc. Add a measured excessive volume of 0.02 *N* $H_2SO_4$ and continue the evaporation until the odor of $CHCl_3$ has disappeared. Cool the soln and titrate back with 0.02 *N* NaOH, using 1 drop of methyl red as indicator. 1 cc of 0.02 *N* $H_2SO_4 = 5.784$ mg of atropine or 6.945 mg of atropine sulfate.

## CINCHONA ALKALOIDS

### QUININE, CINCHONIDINE, CINCHONINE, AND QUINIDINE[18]—TENTATIVE

### 40 REAGENTS

(a) *Sulfuric acid.*—0.225 *N*. Dilute 225 cc of normal $H_2SO_4$ to 1000 cc.

(b) *Sulfuric acid.*—To 93 cc of $H_2O$ add 7 cc of $H_2SO_4$.

(**c**) *Acidified Rochelle salt soln.*—To each 100 cc of a saturated soln of Rochelle salt add 3 cc of (**a**).

(**d**) *Washing soln.*—Dilute (**c**) with an equal volume of $H_2O$.

## 41 DETERMINATION

Weigh a quantity of the sample sufficient to give approximately 0.5 g of total alkaloids; dissolve in the dilute $H_2SO_4$; filter if necessary; add an excess of $NH_4OH$; and extract with $CHCl_3$ until the alkaloids are completely removed. Evaporate the combined $CHCl_3$ extractions on a steam bath; add 5 cc of absolute alcohol and again evaporate; dry at 110°; and weigh.

Dissolve the residue in 50 cc of the 0.225 $N$ $H_2SO_4$, heat on a steam bath for 10 min., and make just alkaline (shown by a faint permanent precipitate) with a 5% soln of NaOH added cautiously and with stirring. Add sufficient of the 0.225 $N$ $H_2SO_4$ to clear the soln and then add 5 cc in excess. Add 25 cc of the acidified Rochelle salt soln and stir to start precipitation. Remove from the steam bath and place in an ice box at 10–15°, stirring occasionally for 2 hours. Filter, and wash with 40 cc of the cold washing soln, using a small wash bottle and stirring the precipitate on the filter with a rubber-tipped glass rod to remove all soluble alkaloidal salts. Designate the combined filtrate and washings as soln "*A*" and save for determination of quinidine and cinchonine. Decompose the precipitate of quinine and cinchonidine tartrates with the warm dilute $H_2SO_4$ and transfer to a separator, washing the filter thoroly to remove all alkaloids. Make alkaline with $NH_4OH$ and remove alkaloids completely by successive extractions with $CHCl_3$. (Four extractions with 25, 20, 15, and 10 cc portions, respectively, are usually sufficient.) Evaporate the combined $CHCl_3$ extractions in a weighed beaker containing a little sharp sand, add 5 cc of absolute alcohol, evaporate to dryness on a steam bath, heat for 3 hours at 100°, cool, and weigh the mixed anhydrous alkaloids of quinine and cinchonidine. Add exactly 1 cc of the 0.225 $N$ $H_2SO_4$ for each 0.015 g of alkaloids and when completely dissolved transfer the soln to a polariscope tube, filtering if necessary. Use the longest tube possible, reducing its capacity when only small quantities of soln are available by inserting a straight tube of small bore slightly shorter than the polariscope tube and fastened securely as to center. Use a bichromate filter and correct the instrument for the operator's eyes. Great precision is necessary for accurate determinations. Read at 20° and calculate to a basis of a 100 mm tube. If the Ventzke scale is used, calculate to angular degrees by multiplying the reading by the factor 0.34657.

(**a**) *Quinine.*—Calculate the percentage of quinine in the total anhydrous alkaloids obtained in the tartrate separation from the following formula, which is based on the specific rotations of quinine (−277.4°) and cinchonidine (−180°) and the observed rotation of the mixed alkaloids at 20° calculated to a 100 mm tube.

$Q = (-68.44)\ (a + 2.7)$, in which

$Q$ = percentage of quinine in the total anhydrous mixed alkaloids obtained in the tartrate separation; and

$a$ = the observed angular rotation, calculated to a 100 mm basis.

(**b**) *Cinchonidine.*—Determine cinchonidine by difference.

Calculate these results to percentage basis of original sample.

(**c**) *Quinidine.*—Place soln $A$ on a steam bath for 10 min., add 0.5 g of KI, remove from the steam bath, and place in the ice box at 10–15° for 2 hours, stirring occasionally. Filter on a weighed Gooch crucible and wash with 15 cc of ice $H_2O$, saving the filtrate and washings (soln $B$). Dry the precipitate of neutral quinidine hydriodide at 100° for 1 hour (a slight yellowing does not affect the results), cool, and weigh. The weight of quinidine hydriodide multiplied by 0.714 gives the weight of anhydrous quinidine alkaloid.

(d) *Cinchonine.*—Transfer soln *B* to a separator, make alkaline with strong $NH_4OH$, and extract completely with $CHCl_3$. Combine the $CHCl_3$ extractions in a weighed beaker containing a trace of sharp sand, add 5 cc of absolute alcohol, evaporate to dryness, heat at 100° for 1 hour, cool, and weigh as anhydrous cinchonine alkaloid.

**42** *Constants of Cinchona Alkaloids*

| ALKALOID | SPECIFIC ROTATION IN ABSOLUTE ALCOHOL | APPROXIMATE SOLUBILITY IN ETHER | THALLEIOQUIN TEST* |
|---|---|---|---|
| Quinine | levo 168° | 1 g in 19 cc | Positive |
| Quinidine | dextro 256° | 1 g in 67 cc | Positive |
| Cinchonine | dextro 225° | 1 g in 526 cc | Negative |
| Cinchonidine | levo 108° | 1 g in 188 cc | Negative |

* *Thalleioquin Test:* Add 1 or 2 drops of Br water to 5 cc of an aqueous soln of the alkaloidal salt (1 in 1000) and 1 cc of $NH_4OH$ (10%). The liquid acquires an emerald-green color due to the formation of thalleioquin (U. S. P. X. p. 311).

## 43 EPHEDRA ASSAY[19]—OFFICIAL, FIRST ACTION*

Place 10 g of ephedra, in fine powder, in an Erlenmeyer flask. Add exactly 100 cc of solvent consisting of 3 volumes of ether and 1 volume of $CHCl_3$, cooled to working temp. after being mixed. Stopper securely, shake, and allow to stand at least 5 min. Add 5 cc of 10% $NH_4OH$ and 0.5 g of anhydrous $Na_2CO_3$, stopper tightly, and allow to macerate for at least 4 hours, with occasional shaking. Decant or filter rapidly a 50 cc aliquot of the clear supernatant liquid representing 5 g of the drug, transfer to a separator and shake with 3 portions of 2% $H_2SO_4$, using 15, 10, 10 cc, etc., until the extraction is complete. Combine the acid solns in a separator, neutralize with strong $NH_4OH$, then add about 5 g of anhydrous $Na_2CO_3$, stirring until dissolved. Shake with 5 portions of ether, using 35, 30, 25, 20 and 15 cc, until extraction is complete and combine the ether portions in a second separator. When clear, decant and filter into a small beaker thru a pledget of cotton previously wet with ether.

Evaporate the solvent to 5 cc volume on the steam bath with the aid of the fan, and add bromthymol blue indicator and a measured excess of 0.02 $N$ $H_2SO_4$. Cover with a watch-glass, return to the steam bath in order to dissolve any alkaloid adhering to the sides of the beaker, and then evaporate the ether. Titrate the excess acid with 0.02 $N$ alkali. 1 cc of 0.02 $N$ acid = 0.0033 g of ephedra alkaloids.

## EPHEDRINE IN INHALANTS[20]—TENTATIVE

### 44 REAGENTS

(a) *Sulfuric acid.*—2% soln.

(b) *Washed ether.*—Shake equal volumes of ethyl ether and $H_2O$ in a separator and discard the aqueous layer.

(c) *Strong ammonia.*

(d) *Bromthymol blue indicator.*—0.04% alcoholic soln.

(e) *Sulfuric acid.*—0.02 $N$ soln.

(f) *Distilled water.*—Free from $CO_2$.

(g) *Sodium hydroxide.*—0.02 $N$ soln, free from carbonates.

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* Two procedures for titrating ephedra alkaloids were tentatively adopted. [J. Assoc. Official Agr. Chem., 14, No. 3 (1931)]. When the assay method became official one of the procedures was dropped.

**45** DETERMINATION

Weigh accurately into a small tared beaker, 5–10 g of the sample. Add 10 cc of 2% $H_2SO_4$ stir, allow the mixture to stand about 15 min., and then transfer to a small separator (automatic extractor optional), rinsing the beaker with small portions of ether. Shake gently, and transfer the acid layer to a second separator. Shake with 3 successive 10 cc portions of the $H_2SO_4$ reagent, rinsing the beaker with ether each time. (Test for complete removal of alkaloid.)

Neutralize the combined acid soln with $NH_4OH$, and add 5 cc in excess. Extract the soln with 30 cc of washed ether, transfer the aqueous layer to a second separator, and wash the ether extraction with 1 cc of $H_2O$, adding the washings to the main aqueous soln. Swirl the ether in order to remove $H_2O$ adhering to the side of the separator. After all the $H_2O$ has been removed, filter the mixture into an Erlenmeyer flask through a pledget of cotton wet with ether inserted in a small funnel. Repeat the extraction with liberal portions of ether at least 4 times, or until the alkaloid is removed completely, washing each portion with the same 1 cc of $H_2O$. Evaporate the ether to a volume of 10 cc on a steam bath with moderate heat by the aid of a current of air.

Remove from the bath, and add bromthymol blue indicator and a measured excess of 0.02 *N* $H_2SO_4$. Add about 40 cc of $CO_2$-free $H_2O$, cover with a watch-glass, return to the steam bath in order to dissolve the alkaloid adhering to the sides of the flask, and evaporate all the ether. Titrate the excess acid with 0.02 *N* NaOH, using standard indicator, pH 6.0, for comparison. 1 cc of 0.02 *N* acid = 0.0033 g of ephedrine alkaloid.

### EPHEDRINE IN TABLETS[21]—TENTATIVE

**46** REAGENTS

(a) *Ammonium hydroxide.*

(b) *Washed ether.*—Shake equal volumes of ethyl ether and $H_2O$ in a separator and discard the aqueous layer.

(c) *Bromthymol blue indicator.*—0.04% alcoholic soln.

(d) *Distilled water.*—Free from $CO_2$.

(e) *Sulfuric aid.*—0.02 *N*.

PREPARATION OF SAMPLE

To determine the average weight per tablet, count and weigh 100 tablets or a number representative of the lot.

**47** DETERMINATION

Weigh not less than 20 tablets. Powder in a mortar, weigh accurately a quantity equal to about 2 grains of the alkaloidal salt, and transfer to a separator. Dissolve in the minimum quantity of $H_2O$, then add 5 cc of $NH_4OH$.

Extract the soln with 30 cc of washed ether. Transfer the aqueous layer to a second separator. Wash the ether extraction with 1 cc of $H_2O$, adding the washings to the main aqueous soln. Swirl the ether in order to remove $H_2O$ adhering to the side of the separator. After all the $H_2O$ has been removed, filter into a beaker through a pledget of cotton wet with ether inserted in a small funnel. Repeat the extraction with liberal portions of ether at least 4 times, or until the alkaloid is removed completely, washing each portion with 1 cc of $H_2O$. Evaporate the ether to a volume of 10 cc on a steam bath with moderate heat before a fan and proceed as directed under **45**, beginning with "Remove from the bath and . . ." to end of paragraph. 1 cc of 0.02 *N* acid = 4.03 mg of ephedrine hydrochloride, = 4.28 mg of ephedrine sulfate, and = 3.30 mg of ephedrine.

### 48 QUALITATIVE TESTS FOR EPHEDRINE BY COPPER SULFATE—SODIUM HYDROXIDE[21]—TENTATIVE

To 1 cc of soln containing at least 5 mg of ephedrine, add 0.1 cc of 10% $CuSO_4$ and 1 cc of 20% NaOH soln. Shake with 2 cc of ethyl ether.

A purple color extracted by ether indicates ephedrine or pseudo ephedrine.

| | | MELTING* POINT°C. | ALPHA 20/D | SOLUBILITY |
|---|---|---|---|---|
| Ephedrine | $C_{10}H_{15}NO$ | 39–40 | − 6.3 | Water |
| Pseudo ephedrine | $C_{10}H_{15}NO$ | 117–118 | +51.2 | Cold water (sparingly) |
| Ephedrine hydrochloride | $C_{10}H_{15}NO\ HCl$ | 214–216 | −35.5 | Water |

## METHENAMINE (HEXAMETHYLENETETRAMINE) IN TABLETS[22]—TENTATIVE

### 49 REAGENTS

*Modified Nessler's reagent.*—(1) Dissolve 10 g of $HgCl_2$, 30 g of KI, and 5 g of acacia in 200 cc of $H_2O$, and filter through a pledget of cotton; (2) dissolve 15 g of NaOH in 100 cc of $H_2O$.

### 50 PREPARATION OF SAMPLE

Ascertain the weight of 20 or more tablets, triturate in a mortar to a fine powder, and keep in a small capsule tightly closed with a cork or glass stopper.

### 51 DETERMINATION

Weigh 0.5 g of the powdered product on a metal scoop or watch-glass, transfer to a round-bottomed flask, and add $H_2O$ to a total volume of 100 cc and finally 25 cc of HCl (1+2.5). Connect with a reflux condenser (preferably of the worm type) and boil gently for 15 min. Cool; wash the condenser tube with a little water; and transfer the contents of the flask quantitatively to a 250 cc volumetric flask, finally diluting to the mark with $H_2O$. Chill 30 cc of the modified Nessler's reagent prepared by mixing 20 cc of soln (1) with 10 cc of soln, (2) in a 200 cc Erlenmeyer flask and add a 10 cc aliquot of the hydrolized soln of the sample. Wash down the neck of the container with a jet of water and allow to stand for at least 1 min. Add 10 cc of acetic acid (1+1.5) in such a manner that the inside of the neck is completely washed by the reagent, mix quickly and thoroly by rotating and tilting the flask, and immediately add from a buret 20 cc of 0.1 *N* I soln. Titrate with 0.1 *N* $Na_2S_2O_3$ soln, adding 5–10 drops of starch indicator [**VI–3** (e)] toward the end of the operation, to the disappearance of the blue coloration. The final color of the soln is a pale straw-green. If preferred, the end point may be determined by the reappearance of a faint blue coloration by the addition of a drop of the I soln. 1 cc of 0.1 *N* I soln = 0.001168 g of methenamine.

## METHYLENE BLUE (METHYLTHIONINE CHLORIDE)[23]—OFFICIAL

### 52 PREPARATION OF SAMPLE

(a) *Tablets.*—Weigh separately at least 20 tablets to ascertain the variation in weight. Weigh collectively all unbroken tablets and calculate the average weight per tablet. Powder finely in a mortar at least 10 tablets or 5 g of methylene blue and protect from moisture in a weighing bottle.

(b) *Capsules.*—Count and weigh a representative number of capsules and ascertain the gross weight per capsule. Open the capsules and transfer as much as possible of the contents to a weighing bottle. Deduct the weight of the cleaned, empty capsules from the gross weight and calculate the average net contents. To clean the gelatine capsules cut in two if necessary and wash by agitating with alternate portions of alcohol and ether. Repeat until thoroly clean, finally removing the ether before a fan or air blast. A few drops of glacial acetic acid mixed with the alcohol aids in the cleaning.

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* The melting point determination for ephedrine is a tentative method.

## **53** PREPARATION OF SOLUTION

(**a**) *Foreign material absent.*—Weigh into a 50 cc beaker 100–140 mg of the sample prepared as directed under **52** and transfer to a 200 cc volumetric flask with 100–140 cc of $H_2O$. Dissolve completely by heating on a steam bath, with frequent shaking, for 30 min.

(**b**) *Oils or water-insoluble material present.*—Transfer to a 150 cc beaker a weighed quantity of the sample, prepared as directed under **52**, corresponding to 100–140 mg of methylene blue. Add 15 cc of $CCl_4$, warm on a steam bath a few min., and stir with a glass rod to dissolve the oils. Transfer to a 100 cc separator, using about 50 cc of hot $H_2O$ and a little $CCl_4$ if necessary. Cool, shake, and allow to separate. Transfer the $CCl_4$ with the undissolved material into a second separator for further treatment. (A clear aqueous soln of the dye should now remain in the first separator. If not clear, extract with another 15 cc portion of $CCl_4$, transferring in a similar manner any remaining insoluble material to the second separator.) Add about 10 cc of $CCl_4$ to the second separator and remove the methylene blue by shaking vigorously with 20–40 cc portions of $H_2O$ until practically no more dye is extracted. (A few drops of glacial acetic acid hastens this extraction.) To the aqueous extracts in a 400 cc beaker add the main soln from the first separator, cover with an inverted watch-glass on glass rods, and evaporate to a volume of about 50 cc. Proceed as directed under (**c**). The $CCl_4$ soln may be reserved for qualitative tests for oils if desired.

(**c**) *Water-soluble material present.*—Use either the aqueous soln from (**b**), or a weighed portion of the sample corresponding to 100–140 mg of methylene blue. Dissolve completely by heating on a steam bath in a 150 cc beaker with about 50 cc of $H_2O$ for 30 min., shaking occasionally. Transfer to a 100 cc separator, keeping the volume as small as possible. Extract with dichlorhydrin, using 10, 5, 3, and 2 cc portions. Combine the dichlorhydrin extracts in a 200–300 cc separator, add 3 or 4 times their volume of $CCl_4$, and extract the dye with $H_2O$ by repeated vigorous shaking with 30–50 cc portions. A few drops of glacial acetic acid hastens the removal. From the combined aqueous extracts remove any traces of dichlorhydrin by shaking once with about 15 cc of $CCl_4$, which is drawn off after settling for 5–10 min. Evaporate the aqueous extracts to about 50 cc over a flame, covering the beaker as in (**b**) with an inverted watch-glass. Transfer to a 200 cc volumetric flask. Dissolve completely by heating on a steam bath with frequent shaking for 30 min.

## **54** DETERMINATION

Conduct a blank in the same manner as the determination, including the filtration. Cool the soln from **53** (**a**) or (**c**), add 50 cc of glacial acetic acid, shake thoroly, and allow to stand at least 25 min. Add from a buret a total of 30 cc of 0.2 $N$ I soln, adding the first 10 cc by fast drops with constant rotating of the flask and the remaining 20 cc at full speed, and continue the shaking. Stopper the flask and allow to stand 50 min., shaking thoroly 5 or 6 times during the interval. Dilute to the mark with $H_2O$, shake, and let stand 10 min. longer. Filter rapidly thru a dry, folded, 12 cm filter paper. Titrate a 100 cc aliquot with 0.1 $N$ $Na_2S_2O_3$ soln with or without starch indicator, as desired. Correct for the number of cc required to titrate the blank run in the same way. 1 cc of 0.2 $N$ I soln = 0.01495 g of methylene blue ($C_{16}H_{18}N_3ClS.3H_2O$).

### 55 CAMPHOR[24]—OFFICIAL, FIRST ACTION

Weigh accurately into a 400 cc round-bottomed Pyrex flask, a sufficient quantity of the powdered substance to contain approximately 2 g of camphor. Add 10 cc of benzol and 10 cc of $H_2O$ and connect the flask with an apparatus for steam distillation. Use an 8–12 inch bulb condenser, well cooled, the outlet of which reaches to the bottom of a 200 cc flask. Distil with steam, collecting the benzol and about 100 cc of aqueous distillate. Disconnect the condenser and wash it slowly with 5 cc of alcohol from a pipet in such a manner as entirely to wet the inside of the condenser. Wash the condenser in the same manner with 10 cc of benzol. Add both washings to the contents of the receiver. Saturate the distillate with NaCl, add sufficient $H_2SO_4$ (1 + 9) to insure acidity, transfer to a separator, shake, and separate the two layers. Rinse the original receiver with 10 cc of benzol and with the rinsings re-extract the aqueous soln. Separate the aqueous layer and extract it once more with 10 cc of benzol. Wash the combined benzol portions with 10 cc of saturated salt soln rendered distinctly alkaline with $Na_2CO_3$. Separate the layers and extract the aqueous layer with 10 cc of benzol. Discard the aqueous solns, transfer the benzol portions to a 50 cc volumetric flask, and make up to the mark with benzol. Shake the soln and filter it into a 200 mm polariscope tube, using a water-jacketed tube, if necessary, in order to maintain a constant temp. of 20°. Make 10 readings, using a bichromate filter, and take the average reading for calculating the camphor. Calculate the quantity of camphor ($Q$) contained in the 50 cc of benzol and, therefore, in the sample taken, from the average reading in circular degrees ($a$) by the following formula:

$$Q = 0.6171\ a - 0.0022\ a^2.$$

The value of $Q$ does not vary directly with the length of the tube. If a longer or shorter tube than directed is used, the value of $a$ must be corrected to a 200 mm tube, and the calculation then made in accord with the above formula.[25]

## MONOBROMATED CAMPHOR IN TABLETS

### *Method I[26]—Official*

### 56 REAGENT

*Sodium amalgam.*—Cut about 1 g of bright metallic Na into small pieces. Dissolve these in 100 g of warm Hg, contained in a small porcelain mortar, by impaling the pieces successively on the point of a file and holding them submerged in the Hg until the rather violent action is complete. Keep the resulting amalgam in a tightly corked bottle.

### 57 PREPARATION OF SAMPLE

Count and weigh a suitable number of tablets to ascertain the average weight; reduce them to a fine powder and keep it in a tightly stoppered bottle.

### 58 DETERMINATION

Weigh a portion of the powdered sample corresponding to 0.1–0.2 g of monobromated camphor, and transfer quantitatively with 20 cc of alcohol and 10 cc of $H_2O$ to a small (100 cc) round-bottomed flask containing 15 g of the Na-amalgam.

Connect the flask, by means of a rubber stopper, with a vertical condenser. Boil the mixture gently over a wire gauze for a period of not less than 30 min. Cool slightly and wash out the condenser tube with 5 cc of alcohol and 5 cc of $H_2O$, receiving the washings in the flask. Place the flask on a steam bath and heat for another hour, or until the evolution of H has nearly or quite ceased. Toward the latter part of this operation, to facilitate the reduction, render the liquid about neutral with a few drops of strong acetic acid. Transfer the contents of the flask to a separator, preferably of the Squibb type, withdrawing the Hg into a second separator and washing it with at least two 50 cc portions of $H_2O$. Pass the several aqueous solns quantitatively thru a small filter, collecting the clear filtrate in a suitable beaker, precipitate with 10% $AgNO_3$ soln, add about 5 cc of $HNO_3$, and filter, collecting the AgBr on a weighed Gooch crucible; wash with $H_2O$ and alcohol; dry at 100°; and weigh. The weight of AgBr multiplied by 1.23 gives the quantity of monobromated camphor originally present in the portion taken for analysis. A control should be run on the amalgam in order to determine whether any correction is necessary for the presence of halogens.

### *Method II*[27]*—Tentative*

### 59 REAGENTS

(a) *Standard alcoholic potassium hydroxide soln.*—0.5 *N* (U. S. P.).
(b) *Alcoholic silver nitrate soln.*—Dissolve 0.2 g of $AgNO_3$ in 50 cc of alcohol.

### 60 PREPARATION OF SAMPLE

Proceed as directed under 57.

### 61 DETERMINATION

Weigh in a small beaker a quantity of the powdered sample equivalent to about 0.2 g of monobromated camphor, add 25 cc of alcohol, warm on a steam bath, and filter into a flask (preferably about 250 cc capacity and provided with a ground-in condenser), washing both beaker and filter with warm alcohol. Add 50 cc of the standard alcoholic KOH soln and 25 cc of the alcoholic $AgNO_3$ soln, and connect with a reflux condenser. Boil gently 1.5 hours, adding at intervals thru the condenser 25 cc more of the alcoholic $AgNO_3$ soln. Cool, and transfer the contents of the flask to a large evaporating dish. Dilute to 200 cc and decant into a beaker, washing the sediment of $Ag_2O$ with $H_2O$ by decantation. Boil the soln 5 min. with 1 g of Zn dust to clarify; filter into another beaker, washing thoroly with $H_2O$; and add $HNO_3$ to decided acidity and 0.1 *N* aqueous $AgNO_3$ soln to complete precipitation. When the AgBr has agglutinated, filter on a weighed Gooch crucible; wash with $H_2O$ and alcohol; dry at 100°; and weigh. The weight of AgBr multiplied by 1.23 gives the quantity of monobromated camphor originally present in the sample taken for analysis. A control should be run on the reagents used in order to determine whether any correction is necessary for the presence of halogens.

## NITROGLYCERIN[28]—OFFICIAL, FIRST ACTION

### 62 REAGENTS

(a) *Ethyl ether.*—U. S. P.
(b) *Ethyl alcohol.*—95% by volume.
(c) *Distilled water.*—Recently boiled and cooled and $NH_3$-free.
(d) *Devarda alloy.*
(e) *Aluminum wire.*—Heavy, about No. 16 gage.
(f) *Alcoholic potassium hydroxide.*—Dissolve 15 g of KOH in ethyl alcohol and dilute with alcohol to 100 cc.

(g) *Standard acid soln.*—0.02 *N* HCl or 0.02 *N* $H_2SO_4$.

(h) *Sodium hydroxide soln.*—0.02 *N*.

(i) *Methyl red indicator.*—Prepare as directed under **38** (e).

**63** APPARATUS

(a) *Kjeldahl distillation flask.*—800 cc capacity.

(b) *Connecting bulb.*—Hopkins style, about 7.6 cm (3 inches) in diameter. This style has a long inlet tube with an opening on the side of the tube.

(c) *Condenser.*—Water-cooled, length about 56 cm (22 inches), and preferably of Pyrex glass.

(d) *Adapter tube.*—About 2.25 cm ($\frac{7}{8}$ inch) in diameter at the top and with narrow outlet.

(e) *Scrubber-trap.*—Any efficient trap in which all the vapor is washed thoroly with $H_2O$ before it leaves the distilling flask.

DETERMINATION

**64** *Method I*

Place in a 50 cc beaker a sufficient quantity of the sample, accurately weighed, to yield about $\frac{1}{2}$ grain (0.0324 g) of nitroglycerin. (If the sample consists of tablets, count those taken; if of powdered material, mix thoroly before weighing the portion taken for analysis.) Add 10 cc of ether, and to facilitate extraction reduce the tablets to a fine powder by means of a glass stirring rod having a flattened end. After stirring thoroly, decant the ether thru a dry 7 cm quantitative filter paper into a 250 cc beaker containing 10 cc of alcohol. Hold the filter paper in place in the funnel with the stirring rod and pour the ether down the rod. Make four additional extractions in the same way. Dissolve the ether-insoluble residue in a small quantity of $H_2O$, transfer the soln to a separator, and extract it twice with 10 cc portions of ether. Filter these extracts, add them to the first extracts, and to remove most of the ether evaporate the combined solns to a volume of about 10 cc by means of an air current from an electric fan.

Transfer the alcoholic soln containing the nitroglycerin to an 800 cc Kjeldahl flask, rinsing the beaker first with 10 cc of alcohol and then with a little $H_2O$. Dilute to about 300 cc with the $NH_3$-free $H_2O$ and place the flask on a wire gauze with an asbestos center. Add 2 g of Devarda alloy (by means of a funnel), about 4 cm of the Al wire, and 10–15 cc of the alcoholic KOH soln. Immediately after adding the alkali, place a little $H_2O$ in the scrubber trap and insert into the flask the rubber stopper carrying the connecting bulb and trap. Connect the outlet tube of the connecting bulb with the water-cooled condenser, which has been fixed in an upright position, fitted with the adapter dipping to the bottom of a 500 cc Erlenmeyer flask containing a measured volume (about 25 cc) of 0.02 *N* acid and 10–15 cc of $H_2O$, and inclined in such a way that the tip of the adapter is submerged as far as practicable under the surface of the liquid in the flask. Heat the distillation flask for about 1 hour, using a small flame and regulating the heat applied so that rapid evolution of H—but no appreciable distillation—takes place. Then gradually increase the heat until distillation begins; when active foaming ceases, continue the distillation with a large flame until all but about 40 cc of the liquid in the distilling flask has distilled over. Lower the flame toward the end of the distillation to avoid cracking the flask. Remove the receiver containing the distillate, add sufficient methyl red indicator to make the soln red, and titrate the excess of acid with 0.02 *N* NaOH soln. From the difference between this excess and the quantity added, after making such correction as may be shown to be necessary by a blank test with the same quantity of reagents and distilled in the same manner, calculate the percentage of nitroglycerin in the sample. 1 cc of 0.02 *N* acid neutralized by the ammonia = 0.001514 g of nitroglycerin.

### 65 *Method II*

Place in a glass-stoppered Erlenmeyer flask a sufficient quantitiy of the sample, accurately weighed, to yield about 1 grain (0.0648 g) of nitroglycerin. (If the sample consists of tablets, count those taken; if of powdered material, mix thoroly before weighing the portion taken for analysis.) Add 50 cc of 95% alcohol by means of a pipet. To facilitate extraction reduce the tablets to a fine powder with a glass stirring rod flattened at one end. Stopper the flask and shake. Allow the mixture to settle, transfer a 25 cc aliquot of the clear soln to an 800 cc Kjeldahl distilling flask, dilute to about 300 cc with the $NH_3$-free $H_2O$, and proceed as directed in **64**.

## MERCURIC IODIDE IN TABLETS[29]—TENTATIVE

### 66 REAGENT

*Standard potassium iodate.*—Dissolve 3 g of pure $KIO_3$, previously dried at about 120° in $H_2O$ and dilute to 1 liter. 1 cc = 0.00637 g of $HgI_2$.

### 67 DETERMINATION

Powder and mix a representative number of the tablets. Weigh accurately a sufficient quantity of the powdered material to represent 1–2 grains of $HgI_2$. Transfer to a 300 cc glass-stoppered Erlenmeyer flask. To the flask add a cooled mixture of 30 cc of strong HCl, 20 cc of $H_2O$, and about 5 cc of $CHCl_3$. Rotate the flask to disintegrate the powder and dissolve the $HgI_2$.

Titrate with the standard $KIO_3$, adding the soln rapidly while rotating the flask. When the I that is liberated during the first stage of the reaction has disappeared from the soln, insert the stopper and shake vigorously for about 30 seconds. Continue the titration slowly, shaking thoroly after each addition, until the I color just disappears from the $CHCl_3$, marking the end point.

The following equation represents the completed reaction:

$$HgI_2 + KIO_3 + 6HCl \rightarrow HgCl_2 + KCl + 3ICl + 3H_2O.$$

The reaction occurs in two stages, in the first of which I is liberated. This is represented as follows:

$$(1) \quad 2HgI_2 + KIO_3 + 6HCl \rightarrow 2HgCl_2 + KCl + 3H_2O + ICl + 2I_2.$$

The I so liberated reacts with additional $KIO_3$ as follows:

$$(2) \quad 2I_2 + KIO_3 + 6HCl \rightarrow KCl + 5ICl + 3H_2O.$$

## OPIUM ALKALOIDS

## MORPHINE IN TABLETS[30]

### 68 *Qualitative Tests—Official*

(1) To the residue or tablet add $HNO_3$. An orange-red color fading to yellow is produced.

(2) To an aqueous soln add a few drops of a 10% $K_3Fe(CN)_6$ soln and then a drop of a 10% $FeCl_3$ soln. A deep blue soln results; a blue precipitate separates on standing.

(3) See Microchemical Tests, **33–36.**

### *Quantitative Method—Official*

### 69 REAGENTS

(a) *Alkaline salt soln.*—Dissolve 30 g of NaOH in $H_2O$, dilute to 1 liter, add NaCl to saturation, and filter.

(b) *Chloroform-alcohol solvent.*—Mix 90 cc of $CHCl_3$ with 10 cc of alcohol.

(c) *Methyl red indicator.*—Prepare as directed under **38** (e).

### 70 PREPARATION OF SAMPLE

To ascertain variation in weight, weigh separately at least 20 tablets. Also weigh collectively a representative number of unbroken tablets and calculate the average weight per tablet. To insure representative sampling in tablets containing more than ¼ grain of alkaloid, pulverize about 20 tablets, mix the powder thoroly, and protect it from moisture in a weighing bottle.

### 71 DETERMINATION

Transfer to a small separator a sufficient number of the tablets, or powdered material equal to a multiple of the average weight per tablet, to represent not to exceed 150 mg of the alkaloid. Moisten with 5 cc of $H_2O$, shake gently, and then dissolve completely by adding 10 cc of the alkaline salt soln. To the alkaline salt soln, add a small piece of litmus paper and then HCl, dropwise, until it is neutral. Add 10 drops in excess. Add 5 cc of alcohol; carefully neutralize with $NH_4OH$ dropwise, and then add 5 drops in excess. Invert the separator and open the stopcock to insure neutralization of residual acid. Immediately extract, at least 6 times, with the $CHCl_3$-alcohol solvent, using 30, 20, 20, 10, 10, and 5 cc, or until the alkaloid is completely removed. (Test for the complete extraction of the alkaloid. Make an additional extraction with 10 cc of the $CHCl_3$-alcohol solvent; evaporate the solvent in a separate beaker; dissolve the residue in a few drops of methyl alcohol; add a drop of methyl red; and dilute with 20 cc of $H_2O$, carbonate free. A yellow color indicates incomplete extraction. Titrate, and add the quantity thus obtained to the total.) Combine the $CHCl_3$-alcohol extractions in a second separator, into the stem of which is inserted a pledget of cotton wet with $CHCl_3$. Wash the combined extractions with 1 cc of $H_2O$. When clear, filter into a small beaker. Extract the wash $H_2O$ twice with small portions of the $CHCl_3$-alcohol solvent. Evaporate on a water bath, using an electric fan to prevent decrepitation of the residue. When dry, remove immediately and complete the determination by one of the following procedures:

(1) To the alkaloidal residue add 2–3 cc of methyl alcohol; cover the beaker with a watch-glass and heat on a steam bath until the residue, including any portions thereof that may adhere to the upper part of the beaker, is completely dissolved. Add 2 drops of the methyl red indicator and, without dilution with $H_2O$, titrate carefully with 0.02 $N$ $H_2SO_4$ to a faint pink, avoiding an excess. Cover the beaker and digest on a steam bath until all particles are completely dissolved. If more than 2 cc of alcohol is added, evaporate the excess. Cool, and dilute with 50 cc of boiled $H_2O$. (The soln should now be yellow.) Finish the titration with the standard acid to a faint red.

(2) Dissolve the residue with 2–3 cc of methyl alcohol on a steam bath. Add 2 drops of the methyl red indicator and then add from a buret 5–10 cc excess of 0.02 $N$ $H_2SO_4$, noting the total quantity used. Cover the beaker with a watch-glass and heat on a steam bath until the residue, including any portions thereof that may adhere to the upper part of the beaker, is completely dissolved. Dilute with 50 cc of cold, previously boiled $H_2O$. Titrate back with the 0.02 $N$ NaOH soln. The $H_2O$ and

alkali should be sufficiently free from carbonates to insure a sharp end point with methyl red. 1 cc of 0.02 *N* acid = 7.513 mg of morphine hydrochloride ($C_{17}H_{19}O_3N\text{-}HCl.3H_2O$) or 7.587 mg of morphine sulfate [$(C_{17}H_{19}O_3N)_2H_2SO_4.5H_2O$].

Alkaloids other than morphine are extracted by $CHCl_3$, while morphine remains in the fixed alkali soln. In general, this separation is unnecessary. If the tablets are of unknown composition or atropine or hyoscine is present, shake the alkaline salt soln with 10 cc portions of washed $CHCl_3$ (use ether for the separation of atropine). Transfer the clear solvent to a small beaker and evaporate on a steam bath. If a residue is obtained, apply the usual tests.

### 72 APOMORPHINE IN TABLETS[31]—TENTATIVE

Weigh a number of tablets equivalent to about 0.065 g (1 grain) of the alkaloid or of its salt and dissolve in 10 cc of $H_2O$ in a separator. Add 1 cc of a freshly prepared saturated soln of $NaHCO_3$ and 25 cc of peroxide-free ether, and shake the mixture. After separation, draw off the lower layer into a second separator and transfer the ethereal layer to a third separator. Extract the mixture in the second separator repeatedly with 15 cc portions of ether until the alkaloid has been completely removed, using the second and first separators alternately for the shaking, and collecting all the ethereal soln in the third. Discard the aqueous soln. Wash the ethereal soln of the alkaloid 3 times with 5 cc portions of $H_2O$, uniting the aqueous washings in a clean separator. Extract these washings with a little fresh peroxide-free ether. Discard the aqueous portion, wash the ether with $H_2O$, discard the washings, and add the washed ether to the main portion of the ethereal soln. Add 20 cc of 0.02 *N* $H_2SO_4$ to the ethereal soln of the alkaloid in the separator and shake the mixture thoroly. Transfer the mixture to a beaker; wash the separator twice with 5 cc portions of $H_2O$, adding the washings to the acid liquid in the beaker; and without delay evaporate the ether at a low temp., preferably on the water bath with the aid of a blast of air. Titrate the excess of acid with 0.02 *N* NaOH, using one drop of methyl red as indicator. 1 cc of 0.02 *N* $H_2SO_4$ = 0.00625 g of apomorphine hydrochloride, $C_{17}H_{17}O_2NHCl + \frac{1}{2}H_2O$.

## CODEINE IN TABLETS[32]

### 73 *Qualitative Tests—Official*

**(a)** To the residue or tablet add strong $HNO_3$. A yellow color is produced.

**(b)** To an aqueous soln add a few drops of a 10% $K_3Fe(CN)_6$ soln and 1 or 2 drops of $FeCl_3$ soln. A green color is produced.

### *Quantitative Method—Official*

### 74 PREPARATION OF SAMPLE

Proceed as directed under **70**.

### 75 DETERMINATION

Transfer to a small separator a sufficient number of tablets, or powdered material equal to a multiple of the average weight per tablet, to represent approximately 150 mg of the alkaloid and dissolve in a minimum of $H_2O$, not to exceed 5 cc, acidified with 2 drops of HCl. Add solid $NaHCO_3$ until neutralized, then a slight excess, and extract 5 times with $CHCl_3$, using 30, 20, 20, 10, and 5 cc. (Test for complete extraction of the alkaloid. Make an additional extraction with 10 cc of $CHCl_3$; evaporate the solvent in a separate beaker; dissolve the residue in a few drops of methyl alcohol; add a drop of the methyl red indicator [**38**(e)]; and dilute with 20 cc of $H_2O$, carbonate free. A yellow color indicates incomplete extraction. Titrate, and add the quantitiy thus obtained to the total.) Combine the $CHCl_3$ extractions in a second separator, into the stem of which is inserted a pledget of cotton wet with chloroform. Wash the combined extractions with 1 cc of water containing 1 drop of $NH_4OH$ and proceed as directed under **71**, beginning with "Evaporate on a water bath." 1 cc of 0.02 $N$ $H_2SO_4 = 7.87$ mg of codeine sulfate $[C_{18}H_{21}O_3N)_2H_2SO_4.5H_2O]$ or to 8.67 mg of codeine phosphate $(C_{18}H_{21}O_3NH_3PO_4.2H_2O)$.

### DIACETYLMORPHINE (HEROIN) IN TABLETS[32]

**76** *Qualitative Tests—Official*

(**a**) Heat about 0.1 g with 1 cc of concentrated $H_2SO_4$ and 1 cc of alcohol. Ethyl acetate, readily recognized by its odor, is formed.

(**b**) Place a fragment of the alkaloid on a glass slide and add 1 drop of 0.1 $N$ HCl. When completely dissolved add a drop of 10% $PtCl_4$ soln and, without stirring or otherwise disturbing the mixture, examine under a low power lens. The precipitate first formed is amorphous, but after a min. or two in the case of pure diacetylmorphine and in 10–15 min. in the case of mixtures, clusters of needles form around a nucleus. Within a short time these needles begin to fly off and continue to do so until the whole cluster is disintegrated.

**77** *Quantitative Method—Official*

Weigh and transfer directly to a small separator a number of tablets representing approximately 150 mg of the alkaloid. Dissolve in 5 cc of $H_2O$ containing 1 drop of strong acetic acid. Add 1 cc of $NH_4OH$ and extract 5 times with $CHCl_3$, using 30, 20, 10, 10, and 5 cc, respectively. Combine the $CHCl_3$ extracts in a second separator, into the stem of which is inserted a pledget of cotton wet with $CHCl_3$. Wash the combined extraction with 1 cc of $H_2O$ and proceed as directed under **71**, beginning with "Evaporate on a water bath." 1 cc of 0.02 $N$ $H_2SO_4 = 8.476$ mg of diacetylmorphine hydrochloride $(C_{21}H_{23}O_5NHCl.H_2O)$.

### PEPSIN IN LIQUIDS[33]—TENTATIVE

**78** REAGENTS

(**a**) *Pepsin.*—Powder a good grade of U. S. P. pepsin and pass it thru a No. 60 sieve, dry in vacuo over $H_2SO_4$ or other suitable desiccating agent, again pass thru the sieve, and preserve in a stoppered bottle in a desiccator. Ascertain the proteolytic power of the dry powder by the U. S. P. method and express it in percentage of the minimum requirement for the U. S. P. product.

(**b**) *Standard pepsin soln.*—Weigh a suitable quantity of the pepsin (**a**) and dissolve in the requisite quantity of 0.1 $N$ HCl to make the soln contain the equivalent of 5 mg of "100%" pepsin per cc. This soln should be freshly prepared.

(**c**) *Dilute standard pepsin soln.*—Dilute 10 cc of the standard pepsin soln (**b**) to 100 cc with 0.1 $N$ HCl. 1 cc contains the equivalent of 0.5 mg of "100%" pepsin.

(**d**) *Ricin soln.*—Grind commercial ricin, similar to the "Ricin acc. to Prof. Jacoby," to a No. 60 powder; mix thoroly; dry; and keep in a desiccator. Digest 1 g of this powder in 100 cc of 5% NaCl soln for an hour at 37.5°, cool, and filter. This soln should be freshly prepared.

**79** PREPARATION OF SAMPLE

(**a**) *Preliminary dilution.*—Determine the acidity of the sample and adjust this to 0.1 $N$ by adding to a measured portion of the sample sufficient 0.2 $N$ HCl or $H_2O$.

Continue the dilution with 0.1 *N* HCl until, upon digestion at 37.5°, 1 cc requires approximately 15 min. to digest the precipitate obtained by mixing 2 cc of the ricin soln and 0.5 cc of 0.1 *N* HCl. (The soln is now about twice the concentration preferred for the final test.) To 50 cc of this soln add 40 cc of 0.1 *N* HCl. Designate this as "preliminary dilution."

(**b**) *Inactivated preliminary dilution.*—Place a portion of the preliminary dilution (1) in a stoppered glass vessel, immerse in a boiling water bath for 15 min., and filter.

(**c**) *Test soln.*—To 18 cc of the preliminary dilution (**a**) add 2 cc of 0.1 *N* HCl. Calculate the proportion of the original sample in this soln.

(**d**) *Inactivated test soln.*—To 18 cc of the inactivated preliminary dilution (**b**) add 2 cc of 0.1 *N* HCl.

(**e**) *Comparison test soln.*—To 18 cc of the inactivated preliminary dilution (**b**) add 2 cc of the standard pepsin solution, **78**(**b**).

## 80 DETERMINATION

Compare the activity of the test soln [**79**(**c**)] with that of the comparison test soln [**79**(**e**)] and that of the dilute standard pepsin soln [**78**(**c**)] as follows:

Arrange 15 test tubes in groups of 5. (A partitioned wire rack is convenient for the purpose.) From the burets introduce into each tube 2 cc of the ricin soln [**78**(**d**)] and 0.5 cc of 0.1 *N* HCl. Place the tubes in a bath maintained at 37.5°; allow sufficient time for the contents of the tubes to reach the temp. of the bath; and add to the tubes the quantities of the solns indicated in the following table, adding the inactive test soln and 0.1 *N* HCl to the tubes first and then as rapidly as possible the active solns, noting the time at which the active solns are added.

| GROUP | | NO. 1<br>*cc* | NO. 2<br>*cc* | NO. 3<br>*cc* | NO. 4<br>*cc* | NO. 5<br>*cc* |
|---|---|---|---|---|---|---|
| I | Test soln **79** (**c**) | 0.00 | 0.25 | 0.50 | 0.75 | 1.00 |
| | Inactivated test soln **79** (**d**) | 1.00 | 0.75 | 0.50 | 0.25 | 0.00 |
| II | Comparison test soln **79** (**e**) | 0.00 | 0.25 | 0.50 | 0.75 | 1.00 |
| | Inactivated test soln **79** (**d**) | 1.00 | 0.75 | 0.50 | 0.25 | 0.00 |
| III | Dilute standard pepsin soln **78** (**c**) | 0.00 | 0.25 | 0.50 | 0.75 | 1.00 |
| | 0.1 *N* HCl | 1.00 | 0.75 | 0.50 | 0.25 | 0.00 |

After the addition of the solns, again immerse the test tubes in the bath. From time to time shake the tubes and observe the progress of the digestions. Note the period required for complete digestion in each tube. The use of a black background facilitates observations.

By comparing any tube of the first group of 5 with the tubes in the remaining groups, the degree of the proteolytic activity of the test soln may be matched against known quantities of standard pepsin both in ordinary acid medium (last group of 5) and in the same medium as the sample itself (second group of 5).

If the rate of digestion is more rapid in the last group than in the second, there is present in the sample some interfering substance that must be removed by dialysis or by evaporation in vacuo at a low temp. until, upon re-examination, the rate of digestion is found to be identical, or nearly so, in each group. Express the result in percentage of "100%" pepsin in the sample.

Smaller quantities of pepsin may be determined in the same way by comparing them with more dilute solns of standard pepsin. Thus 0.05 mg of U. S. P. pepsin can be readily detected by the nearly complete solvent action on the ricin precipitate in less than 2 hours. A marked action on the ricin within the same time is shown by 0.005 mg. For all practical purposes the absence of an appreciable solvent action after 4 hours' digestion indicates the absence of pepsin.

## PHENOLPHTHALEIN IN TABLETS

### *Iodine Method*[34]—*Official*

### 81 REAGENTS

(a) *Potassium hydroxide soln.*—Dissolve 100 g of KOH in an equal weight of $H_2O$.

(b) *Iodine reagent.*—Dissolve 20 g of KI in a minimum quantity of $H_2O$, add 14 g of I, and when dissolved dilute to 120 cc. Add sufficient of the KOH soln to discharge the I color.

(c) *Sodium sulfite soln.*—Dissolve 15 g of $Na_2SO_3$ in $H_2O$ and dilute to 100 cc.

(d) *Hydrochloric acid.*

### 82 PREPARATION OF SAMPLE

Count and weigh a representative number of tablets to ascertain the average weight, grind to a fine powder, and mix thoroly. Weigh out 1 g of the powdered material in a 100 cc volumetric flask; add about 60 cc of alcohol; and boil gently for about 20 min., rotating the flask occasionally. Cool; make up to volume with alcohol; mix thoroly; and filter thru paper, covering the funnel with a watch-glass to avoid evaporation. Pipet one or more aliquots of 10 cc each into 100 cc beakers, or preferably beaker flasks. Evaporate to dryness on a steam bath to remove the alcohol completely.

### 83 DETERMINATION

To the dry residue obtained under **82** add 3 or 4 drops (if fat is present, 1 cc) of the KOH soln and dilute with about 5 cc of $H_2O$ to dissolve the phenolphthalein completely. (As a film of phenolphthalein may be formed on the sides of the beaker during the evaporation of the alcohol, care is necessary to bring the phenolphthalein completely into soln.) Add a piece of ice (about 40 g) and 4–4.5 cc of soln (**b**); then add HCl from a buret, dropwise, stirring the soln with a glass rod, until precipitation is complete; and lastly add KOH soln [**81**(**a**)] from a buret dropwise to dissolve the precipitate completely and consume all the excess of I. (If fat is present, a little undissolved material may remain.) Repeat the process of precipitation with the strong HCl and re-solution with the KOH soln 3 or 4 times, using small quantities of the reagents and adding small pieces of ice to keep the soln cold. Add 1–2 cc of the $Na_2SO_3$ soln and, if necessary to remove fat, filter the ice-cold mixture thru a Gooch crucible into a tall 250 cc beaker, using a bell-jar arrangement and washing several times with water. Acidify the soln with strong HCl, add a few cc in excess, and heat on a steam bath for 20–30 min. Filter the mixture thru a weighed Gooch crucible, and wash the coagulated precipitate several times with water and finally with petroleum ether. Dry the crucible and contents at 130–140° to constant weight. The weight of the precipitate multiplied by the factor 0.3872 gives the weight of phenolphthalein.

### *Ether Extraction Method*[35]—*Official*

(Not applicable in the presence of other alkaline ether extractives.)

### 84 PREPARATION OF SAMPLE

Count and weigh a number of tablets to ascertain the average weight, grind to a fine powder, and mix thoroly.

### 85 DETERMINATION

Weigh a portion of the powdered material representing about 0.1 g of phenolphthalein; transfer to a separator by means of 10 cc of 5% NaOH soln and a little $H_2O$; and extract 3 or 4 times with ether using 25 cc for the first and 20 cc for each subsequent extraction. Transfer the ether extracts into a second separator and wash twice with 5 cc of the 5% NaOH soln. (Substances like quinine, acetanilid, acetphenetidin, as well as any unsaponified fatty material or mineral oil, if present, will be removed by extraction with ether.) Combine the alkaline solns and acidify with HCl; then extract with ether as before, until all the phenolphthalein has been removed, as determined by testing a portion of the soln with NaOH. (Usually 4 or 5 extractions will be sufficient.) Filter the ether extractions into a weighed beaker, evaporate, dry the residue at 100°, and weigh. The residue should be soluble in alcohol, showing absence of most oils. When titrated with 0.1 *N* NaOH, the alcoholic soln should be practically neutral, showing absence of acid extractives, like fatty acids and salicyclic or benzoic acid.

## PHENOLPHTHALEIN IN CHOCOLATE PREPARATIONS[36]—TENTATIVE

### 86 REAGENTS

Use the reagents described under **81**.

### 87 PREPARATION OF ALCOHOLIC EXTRACT

To a 1 g sample in a 50 cc volumetric flask, add about 35 cc of 95% alcohol; boil gently for about 20 min., rotating the flask occasionally; cool and make up to volume with alcohol; mix thoroly; filter thru dry paper, covering the funnel with a watchglass to avoid evaporation; pipet a number of aliquots of 10 cc each into 250 cc beakers; and evaporate to dryness on the steam bath to remove the alcohol completely.

### 88 DETERMINATION

Take up the residue in alkali by moistening with about 1 cc of the strong KOH reagent and add a little $H_2O$. When the residue is completely in soln add a piece of ice, about 40 g; pour in the prepared I reagent (4–4½ cc); and add concentrated HCl from a buret, dropwise, using a stirring rod (beaker is not rotated) to complete precipitation. Then make alkaline, using dropwise a strong KOH reagent from a buret until soln is effected—except for the small quantity of fatty material that remains undissolved. Repeat this process 3 or 4 times. Then add 1 or 2 cc of $Na_2SO_3$ soln, and filter the ice cold mixture thru a Gooch crucible into a tall 250 cc beaker, using a bell jar arrangement and washing several times with distilled $H_2O$. Acidify the filtrate with strong HCl, using a few cc in excess, and heat on the steam bath for 20–30 min. Filter the coagulated precipitate hot thru a weighed Gooch crucible; wash a few times with $H_2O$; and when sucked fairly dry wash several times with petroleum ether. Dry the precipitate in the oven (120–140°) to constant weight. The weight of the precipitate multiplied by the factor 0.3872 gives the weight of phenolphthalein.

## SALICYLIC ACID IN THE PRESENCE OF OTHER PHENOLS[37]—TENTATIVE

### 89 REAGENTS

(a) *U. S. P. ether.*

(b) *Sulfuric acid soln.*—(1+9).

(c) *Sodium bicarbonate soln.*—Saturated.

(d) *Chloroform-ether solvent.*—Mix 2 volumes of $CHCl_3$ with 1 volume of ether.

(e) *Sodium hydroxide.*—0.1 *N*.

(f) *Neutral alcohol.*

(g) *Sodium hydroxide soln.*—4 g to 100 cc.

### 90 PREPARATION OF SAMPLE

*Powders.*—Weigh into a volumetric flask a sufficient quantity of the powder for an aliquot of 25–50 cc to contain approximately 0.13 g of phenol. If acid, make alkaline with dilute NaOH and add 25 cc in excess, fill to mark with $H_2O$, and shake well.

*Liquids.*—For liquid samples, proceed as under **91**.

### 91 DETERMINATION

Transfer to a separator a sufficient quantity of soln to represent about 0.13 g of phenol. Acidify with dilute $H_2SO_4$ and extract with ether, using 20, 15, 15 and 10 cc portions, respectively, until extraction is completed. Combine the ether in a second separator, then shake with $NaHCO_3$ soln, using 15, 15 and 10 cc portions, respectively, and finally shake with 15 cc of $H_2O$. Combine the $NaHCO_3$ soln and the washing and extract the soln with 15 cc of ether. Add the latter to the main bulk of ether and reserve for the phenol determination. Acidify the $NaHCO_3$ soln with HCl. Extract with $CHCl_3$-ether solvent, using 30, 25, 20 and 10 cc, respectively, until the salicyclic acid is completely removed. Filter the solvent into a beaker thru cotton previously saturated with $CHCl_3$. Evaporate to 5 cc on a covered steam bath with the aid of an electric fan, allowing the last 5 cc to evaporate spontaneously. Dissolve the residue in 10 cc of neutral alcohol and titrate with 0.1 *N* NaOH, using phenolphthalein as indicator. 1 cc of 0.1 *N* NaOH = 0.01381 g of salicyclic acid ($C_6H_4$-OHCOOH).

## MENTHOL[38]—TENTATIVE

### 92 REAGENTS

(a) *Acetic anhydride.*

(b) *Anhydrous sodium acetate.*—Powdered.

(c) *Fused calcium chloride.*

(d) *Alcoholic potassium hydroxide.*—0.5 *N*.

(e) *Sulfuric acid.*—0.5 *N*.

(f) *Phenolphthalein.*—Dissolve 1 g in 100 cc of 95% alcohol.

(g) *Sodium carbonate soln.*—Dissolve 12.5 g of $Na_2CO_3 . H_2O$ in sufficient distilled $H_2O$ to make 100 cc.

### 93 DETERMINATION

Weigh 5 g of menthol in an acetylization flask of 100 cc capacity, and add 10 cc of acetic anhydride and 1 g of powdered anhydrous Na-acetate. Boil the mixture gently for 1 hour, cool, and disconnect the flask from the condenser, transferring the mixture to a small separator. Rinse the acetylization flask with 3 successive 5 cc portions of warm distilled $H_2O$ and add the rinsings to the separator. When the liquids have completely separated, remove the aqueous layer, and wash the remaining oil with successive portions of $Na_2CO_3$ soln, diluted with an equal volume of distilled $H_2O$, until the mixture is alkaline to 2 drops of phenolphthalein soln. Dry the resulting oil with fused $CaCl_2$ and filter. Transfer 4–5 cc of the dry acetylized oil to a tared 100 cc Erlenmeyer flask, note the exact weight, add 50 cc of 0.5 *N* alcoholic KOH, connect the flask with a reflux condenser, and boil the mixture on a water bath for 1 hour. Allow the mixture to cool, disconnect the flask from the condenser, and titrate the excess of alkali with 0.5 *N* $H_2SO_4$, using 10 drops of phenolphthalein soln as indicator. Calculate the percentage of menthol by the following formula:

$$\text{Percentage of total menthol} = \frac{A \times 7.811}{B - (A \times 0.021)}.$$

*A* is the result obtained by subtracting the number of cc of 0.5 *N* $H_2SO_4$ required in the above titration from the number of cc of 0.5 *N* alcoholic KOH originally taken; *B* is the weight of acetylized oil taken.

### THYMOL[39]—TENTATIVE

### *Quantitative Method*

94

#### REAGENTS

(a) *Standard bromide-bromate soln* (*0.1 N bromine soln*).—Prepare and standardize as directed under **25(c)**.

(b) *Hydrochloric acid soln.*—(1+1).

(c) *Methyl orange soln.*—0.1 g to 100 cc of $H_2O$.

(d) *Sodium hydroxide soln.*—25%.

(e) *Potassium iodide.*

(f) *Sodium thiosulfate soln.*—0.1 *N*.

(g) *Starch indicator.*—Prepare as directed under **VI, 3(e)**.

95

#### DETERMINATION

Weigh 2 g of pulverized thymol, transfer to a 500 cc volumetric flask, and add 25 cc of the NaOH soln. Agitate until the thymol is dissolved and dilute to mark at 20° with $H_2O$.

*Method I:* Transfer a 25 cc aliquot of the thymol soln to a 250 cc glass-stoppered Erlenmeyer flask, add 20 cc of hot HCl (1+1), and immediately run in 1–3 cc less than the theoretical amount of Br soln. Warm to 70–80°, add 2 drops of methyl orange soln, and titrate slowly with the Br soln, swirling vigorously after each addition. When the red color of the methyl orange is bleached, add 2 drops of the titrating soln, stopper, shake vigorously for 10 seconds, add a drop of methyl orange soln. and again shake vigorously for 10 seconds. Continue the addition of 2 drops of Br soln, shaking until the red color disappears. Then add 1 drop of methyl orange soln; shake vigorously; and if the red color does not disappear, repeat the alternate addition of 2 drops of Br soln and 1 drop of methyl orange soln, shaking after each addition as directed above, until the red color disappears. Calculate the number of cc of Br soln used to percentage of thymol. 1 cc of 0.1 *N* Br soln = 0.003753 g of thymol. Reserve the mixture in the titrating flask for Method II.

96

*Method II:* To the cooled mixture resulting from the titration according to Method I, add 3–5 cc additional Br soln. Stopper, shake, add 1 g of solid KI, wash sides of flask and stopper with distilled $H_2O$, and titrate the I liberated by the excess Br soln with the standard thiosulfate soln, using starch soln as indicator. Calculate the amount of thiosulfate used in terms of Br soln, deduct from the total amount of Br soln added, and calculate to percentage of thymol.

To determine the approximate number of cc of Br soln required for Method I, heat a mixture of a 25 cc aliquot of the sample and 20 cc of HCl (1+1) to about 80° and titrate slowly with the Br soln, swirling vigorously while titrating until a yellow color, permanent for 1 min., appears.

### PYRAMIDON (AMIDOPYRINE)

**97**

#### *Qualitative Tests*[40]*—Official*

(a) Dissolve 0.01 g of the sample in 2 cc of $H_2O$ and add a few drops of strong yellow $HNO_3$ (containing nitrous acid). A purplish blue colored solution is produced.

(b) Dissolve 0.01 g of the sample in 2 cc of $H_2O$ and add 1 cc of a 10% $FeCl_3$ soln. A purple to violet color develops, but it becomes red on the addition of $H_2SO_4$ (1+9).

(c) Dissolve 0.1 g of the sample in 2 cc of $H_2O$ and add a few drops of a 5% $AgNO_3$ soln. After a few seconds a purple to violet color is produced and on standing a deposit of metallic Ag results. (Useful for detecting pyramidon in antipyrin).

(d) Dissolve 0.1–0.2 g of the sample in 2 cc of $H_2O$, add 1 or 2 drops of a 0.2 % soln of $NaNO_2$ and a few drops of $H_2SO_4$ (1+9), and shake for a few seconds. A purplish blue color develops, then gradually disappears, leaving a colorless soln A large excess of $NaNO_2$ should be avoided as it destroys the color. (Useful for detecting antipyrin in presence of pyramidon. On addition of a few more drops of the $NaNO_2$ soln and the dilute $H_2SO_4$ a yellowish green colored soln remains after the disappearance of the purple coloration if antipyrin is present.)

#### *Quantitative Method*[41]*—Official, First Action*

**98**

#### PREPARATION OF SAMPLE

Pulverize the material in a mortar and mix the powder thoroly.

**99**

#### DETERMINATION

Place 1 g of the sample in a 100 cc volumetric flask, add 60 cc of *N* HCl, and shake for several min. to insure complete soln of the pyramidon. Make up to the mark with *N* HCl. Filter, if not clear, thru a dry filter, rejecting the first part of the filtrate. Pipet a 20 cc aliquot portion of the soln, or filtrate, into a separator; make distinctly alkaline with either $NH_4OH$ or with dilute NaOH; and shake out with 20, 15, 10, 10, and 5 cc portions of $CHCl_3$. Combine the $CHCl_3$ extracts in a second separator and wash with 2 cc of $H_2O$. Filter the $CHCl_3$ soln into a weighed beaker thru a pledget of cotton saturated with $CHCl_3$. Extract the wash $H_2O$ with 5 cc of $CHCl_3$ and add this to the combined $CHCl_3$ extracts. Evaporate the united $CHCl_3$ extracts just to dryness on a water bath with the aid of an electric fan and dry the residue in an oven at the temp. of boiling $H_2O$ for 10 min. Cool in a desiccator, and weigh as pyramidon. Identify the pyramidon by means of its melting point and qualitative tests.

### PROCAINE HYDROCHLORIDE[32] (NOVOCAINE)

**100**

#### *Qualitative Tests—Official*

(a) Dissolve 0.1 g of the sample in about 10 cc of $H_2O$. Add 2 cc of a 5% $KMnO_4$ soln. Warm, if necessary. Reduction occurs with evolution of gas having the odor of acetaldehyde. (Distinction from cocaine, which does not readily reduce $KMnO_4$.)

(b) Dissolve about 5 mg of the sample in 3 cc of $H_2O$ and add a few drops of Mayer's reagent [**XXXIII, 20(r)**]. In the case of procaine a white precipitate is formed which dissolves if a few cc of $H_2SO_4$ (1+49) is added. (The precipitates with stovaine and cocaine are not readily soluble in dilute $H_2SO_4$.)

(c) Dissolve about 0.1 g of the sample in 2 cc of $H_2O$. From a buret add 25 cc of 0.1 *N* NaOH. (A white precipitate is formed which dissolves in an excess of the NaOH when heated on a steam bath.) Heat the solution for 25 min. on a steam bath. Upon cooling the soln and extracting with $CHCl_3$, no residue should be obtained upon evaporation of the $CHCl_3$. (Stovaine does not readily hydrolize, and a residue giving an alkaloidal reaction remains upon evaporation of the $CHCl_3$.)

(d) Dissolve 0.1 g of the sample in 5 cc of $H_2O$. Add 2 drops of HCl and 2 drops of $NaNO_2$ soln. Pour the mixture slowly into 10 cc of a soln of 0.2 g of beta-naphthol in 10% NaOH soln. A scarlet red precipitate is formed.

(e) Add 5 drops of 0.1 *N* $KMnO_4$ soln to 0.1 g of procaine in 5 cc of $H_2O$ and 3 drops of HCl. The violet color disappears immediately (distinction from cocaine hydrochloride).

(f) Dissolve about 0.1 g of the sample in 1 cc of $H_2SO_4$. The soln remains colorless, showing absence of organic impurities.

## *Quantitative Methods*

### *Method I—Official*

**101** REAGENTS

(a) *Potassium iodide soln.*—Dissolve 20 g of KI in $H_2O$ and dilute to 100 cc.

(b) *Standard bromide-bromate soln* (0.1 *N bromine soln*).—Prepare as directed under **25**(c) and standardize against 0.1 *N* $Na_2S_2O_3$.

(c) *Starch indicator.*—Prepare as directed under **VI, 3**(e).

**102** DETERMINATION

Dissolve 0.1 g of the sample in 5 cc of $H_2O$ in a 50 cc beaker. Add 25 cc of 0.1 *N* NaOH and heat on a steam bath for 25 min. Cool, and transfer the soln to a 500 cc Erlenmeyer flask having a tightly fitting ground-glass stopper. Add 50 cc of standard bromide-bromate soln, dilute with $H_2O$ to 250 cc, add 10 cc of HCl and stopper the flask immediately to avoid loss of Br. Shake the flask occasionally and allow to stand for 30 min. at room temp., keeping the flask tightly stoppered. (It is necessary that a large excess of Br be present, as shown by a bright yellow color.) Add quickly 10 cc of the KI soln, stopper, and shake the flask. Allow to stand for 15 min., shaking occasionally. Titrate the excess of I with 0.1 *N* $Na_2S_2O_3$ soln, using starch indicator. Titrate to disappearance of the blue color (the blue color reappears on standing and should be disregarded). 1 cc of 0.1 *N* bromide-bromate soln = 4.54 mg of procaine hydrochloride ($C_{13}H_{20}O_2N_2HCl$).

### **103** *Method II—Official*

Weigh a quantity of the powder or the number of tablets equivalent to about 0.2 g of procaine. Dissolve in 10–15 cc of $H_2O$ transfer the soln to a separator, and add about 3 cc of $NH_4OH$. Extract the ammoniacal soln 4 or 5 times with $CHCl_3$, using 15 cc for the first extraction and 10 cc for the subsequent extractions. Filter into a weighed beaker and evaporate the $CHCl_3$ by means of an electric fan, preferably at room temp. (Avoid prolonged heating of the procaine base, as it appears to be slightly volatile at 100°.) Calculate the quantity of procaine in the sample by multiplying the weight of extracted residue by the factor 1.1545. Take up the residue with a slight excess of 0.1 *N* or 0.02 *N* acid. Titrate the excess of acid with 0.02 $N$ NaOH, using methyl red indicator [**38**(e)]. 1 cc of 0.1 $N$ acid = 27.27 mg of procaine hydrochloride ($C_{13}H_{20}O_2N_2.HCl$).

### 104 SANTONIN IN SANTONICA (LEVANT WORMSEED)—TENTATIVE

Extract 10 g of the sample, ground to pass a 30-mesh sieve, in a Soxhlet extraction apparatus for 3 hours with $CHCl_3$. Distil the $CHCl_3$ until 7–8 cc remains, add 100 cc of 5% $Ba(OH)_2$ soln, and heat on a steam bath until the odor of $CHCl_3$ has disappeared. Boil 5 min., cool, and saturate with $CO_2$ (washed thru $NaHCO_3$ soln to remove traces of acid). Filter on a small Büchner funnel, using suction, and wash twice with 10 cc of $H_2O$. Heat the filtrate on a steam bath, add 5 cc of HCl (2+1), and warm 5 min. Cool until lukewarm and extract with 20, 15, and 15 cc of $CHCl_3$, passing the solvent thru a pledget of cotton into a flask. Evaporate to dryness, removing the last traces of $CHCl_3$. Dissolve in 7.5 g (9.5 cc) of absolute alcohol, warming gently if necessary. Add 42.5 cc of $H_2O$ heated to 60–70°, stopper the flask, and allow to cool. Start crystallization at this point by scratching the side of the flask with a rod or by seeding with a minute crystal of santonin. (Solns containing a liberal quantity of santonin, kept in a cool place for 24 hours, have been found in a supersaturated condition when this precaution was not observed.) Maintain the flask and contents at a temp. of 15–17° for 24 hours. Filter and wash at 15–17° with two 10 cc portions of alcohol, 15% by weight. Dry the flask and filter at 100°; dissolve the santonin left in the flask and on the filter in $CHCl_3$; and filter into a weighed beaker. Wash the flask and paper thoroly with $CHCl_3$, evaporate the combined filtrate and washings, dry at 100° to remove all traces of $CHCl_3$, and weigh. To the weight found add 0.04 g for the santonin dissolved in the dilute alcohol and multiply the total by 10 to obtain the percentage of santonin.

### SANTONIN IN MIXTURES AND TABLETS[43]—TENTATIVE

### 105 *Langer's Method* (*Modified*)

Weigh out a sample equivalent to approximately 0.15 g of santonin, and extract with 10, 10, 10, 5 and 5 cc portions of petroleum ether saturated with santonin. (If the sample is fat-free this step may be omitted.) Filter each portion of solvent with the aid of suction to complete dryness thru a Gooch crucible provided with an asbestos mat before following with another portion of fresh solvent. Extract the residue in the solution flask and the crucible with 15, 10, 5 and 5 cc of hot benzene, filtering each portion as before. Evaporate the benzene extract in a tared flask and dry the residue to constant weight at 100°. The weight of the santonin in the flask is equal to the weight of santonin in the sample.

### 106 STRYCHNINE IN TABLETS—OFFICIAL

(Other alkaloids absent)

Weigh 25–100 tablets and transfer directly into a separator. Add 10–20 cc of $H_2O$ and 1 cc of $NH_4OH$ (1+2) and shake gently to disintegrate the tablets. Extract 5 times with $CHCl_3$, using 25, 20, 15, 10, and 5 cc portions, respectively, or until the alkaloid is completely removed. Combine the first two extractions in a second separator, into the stem of which has been inserted a pledget of absorbent cotton wet with $CHCl_3$. Wash with 5 cc of $H_2O$ containing a drop of $NH_4OH$ (1+2). When clear, filter the $CHCl_3$ portion into a small beaker. Wash each successive $CHCl_3$ extract with the same wash water and filter in a similar manner into the main portion, finally washing the outer surface of the stem of the separator with a few cc of $CHCl_3$ and adding this also to the main portion. Evaporate on a steam bath, removing the dish from the bath as the last portions evaporate to avoid decrepitation.

Add 2–3 cc of neutral alcohol, cover beaker, and warm on a steam bath to dissolve the residue. Add a few drops of methyl red indicator [**38**(**e**)] and titrate with 0.02 *N* acid to a faint pink color. Add 50 cc of recently boiled $H_2O$ and continue the titration with the 0.02 *N* acid to a faint red color. 1 cc of 0.02 *N* acid = 6.686 mg of strychnine ($C_{21}H_{22}O_2N_2$), 8.568 mg of strychnine sulfate [$(C_{21}H_{22}O_2N_2)_2H_2SO_4 . 5H_2O$], or 7.946 mg of strychnine nitrate ($C_{21}H_{22}O_2N_2HNO_3$).

### 107 SEPARATION OF QUININE AND STRYCHNINE[44]—TENTATIVE

Make 50 cc of the soln acid with citric acid, add an equal volume of $H_2O$, evaporate to nearly the original volume to remove excess alcohol, cool, and extract with two 15 cc portions of ether to remove oily material. Make the aqueous soln alkaline with $NH_4OH$ and extract the mixed alkaloids in the usual way with a mixture of 2 parts of $CHCl_3$ and 1 part of ether, using 25, 20, 15, 10, and 5 cc portions. Evaporate the $CHCl_3$ and ether in a weighed Erlenmeyer flask or beaker to dryness on a steam bath. Add a little ether and again evaporate to dryness to remove the last traces of $CHCl_3$. Dry at 100° for 1 hour and weigh to obtain the approximate weight of mixed alkaloids.

Dissolve the alkaloidal residue in 50 cc of 10% $H_2SO_4$, add 5 cc of 4% $K_4Fe(CN)_6$ dropwise from a buret, stirring well, and set aside for a few hours or overnight. Filter the resulting precipitate through a small (7 cm) filter and wash 3 times with 3 cc of 5% $H_2SO_4$. Reserve the filtrate for the determination of quinine. Wash the precipitate immediately into a small separator with $H_2O$, transferring the precipitate remaining in the flask to the separator by shaking about 3 times with 3 cc of $NH_4OH$ and a small quantity of $CHCl_3$. Extract the ammoniacal soln of the precipitate with 25, 15, 15, 10, and 5 cc portions of $CHCl_3$. Collect the $CHCl_3$ solns in another separator and extract the alkaloids by shaking with 25, 10, 10, and 5 cc portions of 20% $H_2SO_4$; repeat the precipitation with $K_4Fe(CN)_6$ and the other operations, as above, until the $CHCl_3$ extracts are again obtained, reserving the filtrate for determination of quinine. Evaporate the $CHCl_3$ carefully, adding a little alcohol toward the end to prevent sputtering. Weigh the residue of strychnine after drying it for 1 hour at 100°. (This residue should be nearly white and free from quinine.) Check volumetrically as follows: Dissolve the residue in hot alcohol, add 0.02 *N* $H_2SO_4$ until the soln is acid to methyl red indicator [**38**(**e**)], then add 2 or 3 cc in excess. Evaporate most of the alcohol, cool, and titrate back with 0.02 *N* alkali. 1 cc of 0.02 *N* acid = 6.686 mg of strychnine ($C_{21}H_{22}O_2N_2$) or 8.568 mg of strychnine sulfate [$(C_{21}H_{22}O_2N_2)_2H_2SO_4 . 5H_2O$].

Combine the 2 filtrates from the precipitations with $K_4Fe(CN)_6$ in a separator; make alkaline with $NH_4OH$; and extract with a mixture of 2 parts of $CHCl_3$ and 1 part of ether, using 20, 15, 15, 10, and 5 cc portions of the solvent and observing the usual precaution of washing the stem of the separator with the $CHCl_3$-ether mixture after each extraction. Wash the combined extractions in a second separator with two 5 cc portions of $H_2O$, transfer to a weighed beaker, evaporate to dryness, add a few cc of ether, and again evaporate to dryness to remove the final traces of $CHCl_3$; dry at 120–130°, cool, and weigh as anhydrous quinine. Test the residue qualitatively[45] for quinine or if desired, check the quantity volumetrically as follows: Dissolve the residue in a little alcohol, add 7 drops of methyl red (some analysts prefer brom cresol purple with a yellow endpoint) indicator [**69**(**c**)], then add 0.02 *N* $H_2SO_4$ to a distinct red, and 1 cc in excess. Evaporate the soln to a small volume, cool, allow the quinine sulfate to separate, filter thru a small pledget of cotton in the stem of a funnel, wash with small portions of water, and titrate the combined filtrate and washings with 0.02 *N* alkali. 1 cc of 0.02 *N* $H_2SO_4$ = 6.486 mg of anhydrous quinine ($C_{20}H_{24}O_2N_2$); to 7.567 mg of quinine alkaloid ($C_{20}H_{24}O_2N_2 . 3H_2O$);

to 7.467 mg of quinine sulfate, anhydrous, [$(C_{20}H_{24}O_2N_2)_2H_2SO_4$]; and to 8.728 mg of quinine sulfate [$(C_{20}H_{24}O_2N_2)_2H_2SO_4 . 7H_2O$].

### 108 STRYCHNINE IN LIQUID PREPARATIONS—OFFICIAL

(Other alkaloids absent)

Measure into an evaporating dish 50 cc of the sample, or a quantity sufficient to yield at least 65 mg of the alkaloid, and remove the alcohol by evaporation. Transfer to a separator; add 1 cc of $NH_4OH$; and proceed as directed under **106**, beginning with "Extract 5 times with $CHCl_3$."

## COCAINE[46]

### 109 *Method I—Official, First Action*

Weigh accurately a sufficient quantity of the uniformly mixed sample to represent approximately 0.1–0.2 g of the alkaloid. Transfer to a small separator and dissolve with the minimum quantity of water required for soln. Make the soln slightly alkaline with $NH_4OH$ and extract with successive small portions of peroxide-free ether until the alkaloid is completely removed from the aqueous soln, using Mayer's reagent for the test. Combine the ether extracts, remove the greater part of the ether by evaporation on the steam bath, and allow the remainder of the ether to evaporate spontaneously at room temp. Dissolve the residue in a few cc of neutral alcohol, add 20 cc of 0.05 *N* $H_2SO_4$, and titrate the excess of acid with 0.02 *N* NaOH, using methyl red indicator. Each cc of 0.05 *N* $H_2SO_4$ required for combination with the alkaloid is equivalent to 0.01698 g of cocaine hydrochloride ($C_{17}H_{21}O_4N$-HCl).

### 110 *Method II—Tentative*

Weigh accurately a sufficient quantity of the uniformly mixed sample to represent approximately 0.2 g of the alkaloid. Dissolve in 20 cc of cold $H_2O$, add 2 drops of 10% HCl, and transfer to a separator. Make alkaline to litmus with a freshly prepared saturated soln of $NaHCO_3$ and shake out to exhaustion with petroleum ether (four 20 cc portions are usually sufficient). Run the combined extract thru a plug of absorbent cotton into a separator and wash the cotton with petroleum ether. Add a decided excess of 0.02 *N* $H_2SO_4$, accurately measured, and shake vigorously for several min. Separate the 2 layers and wash the petroleum ether with two 10 cc portions of $H_2O$, adding the washings to the acid soln. Titrate the excess of acid with 0.02 *N* alkali, using methyl red indicator, and reserve the titrated soln for the check determination described below. Each cc of 0.02 *N* $H_2SO_4$ required for combination with the alkaloid is equivalent to 0.006795 g of cocaine hydrochloride ($C_{17}H_{21}O_4$-NHCl).

As a check, add 10 cc of 2.5 *N* NaOH soln to the titrated alkaloidal soln and evaporate on the steam bath to a volume of about 10 cc. Cool, transfer the soln to a separator, and acidify with 10% HCl. Extract the acid soln completely with successive portions of $CHCl_3$. Run the combined extracts thru a plug of absorbent cotton and wash the cotton well with $CHCl_3$. Allow the $CHCl_3$ soln to evaporate spontaneously in a weighed beaker, dry the residue in a vacuum desiccator for 2 hours, and weigh. From the weight of benzoic acid found calculate its equivalent of cocaine hydrochloride. 1 g of benzoic acid = 2.782 g of cocaine hydrochloride. (If desired, the quantity of benzoic acid may be determined by titration.)

### FLUIDEXTRACT OF IPECAC

### *Automatic Extraction Method*[47]*—Tentative*

**111** PREPARATION OF SOLUTION

Pipet 20 cc of the fluid extract into a 100 cc volumetric flask, add approximately 5 cc of $N$ $H_2SO_4$, and with the aid of an air blast evaporate on a steam bath to a volume of about 10 cc. Then, while rotating the flask, add about 30 cc of $H_2O$, cool to room temp., and make up to the mark with $H_2O$. Allow to stand over night and filter through a dry filter, rejecting the first few cc of the filtrate.

**112** DETERMINATION

Measure 20 cc of the filtrate, prepared as directed above (equivalent to 4 cc of fluidextract of ipecac) into an automatic extractor for liquids lighter than $H_2O$, and which has been fitted to a 200 cc Erlenmeyer flask. Add 60 cc of $H_2O$ and 2 cc of 8% $NH_4OH$ soln, and about 50 cc of peroxide-free ether. Shake gently to prevent the deposition of any solid matter on the bottom of the extractor and then add peroxide-free ether until about 75 cc has passed over into the flask. Heat the flask on a steam bath (not electric hot plate) and extract for 2 hours, or until the extraction is complete. Separate the ether from the aqueous layer and add it to the main concentrate in the flask. Evaporate the combined ether extract on a steam bath, add 2–3 cc of absolute alcohol, and repeat the evaporation to remove all traces of $NH_3$. Warm the alkaloidal residue on the steam bath with 2–3 cc of neutral alcohol to insure complete soln. Add 10 cc of 0.1 $N$ $H_2SO_4$, and dilute with about 20 cc of recently boiled, cooled $H_2O$. Titrate the excess of acid with 0.02 $N$ NaOH, using methyl-red as indicator. 1 cc of 0.1 $N$ $H_2SO_4 = 0.024$ g of ether-soluble alkaloids of ipecac.

### **113** *Hand Extraction Method—Tentative*

(This method is sometimes more rapid than the automatic extraction method and yields results almost as high.)

Pipet 20 cc of the filtrate, prepared as directed under **111**, into a separator. Add 2 cc of 8% $NH_4OH$ soln and extract the soln with equal volumes of peroxide-free ether until extraction is completed (at least 8 times), using Mayer's reagent as a test. Wash the combined ether extracts in a second separator with about 10 cc of $H_2O$, and then wash this wash $H_2O$ with a little peroxide-free ether, adding the ether washings to the main soln. Transfer the ether soln to an Erlenmeyer flask (a 200 cc flask is a convenient size), and evaporate the ether on a steam bath with the aid of a blast of air. Add 2–3 cc of absolute alcohol and repeat the evaporation to remove all traces of $NH_3$. Warm the alkaloidal residue with 2–3 cc of neutral alcohol to insure complete soln, and titrate as directed in the automatic extraction method.

## TRAGACANTH

**114** VOLATILE ACIDITY[48]—TENTATIVE

The quantity of volatile (acetic) acidity developed in the acid hydrolysis of gum tragacanth (*Astragalus gummifer* Lab.) affords a valuable index of the purity of this commodity when compared with results obtained by similar treatment of so-called "Indian gum" (*Cochlospernum gossypium* D. C. and *Sterculia urens* Roxb.).

Treat 1 g of the whole or powdered sample in a 700 cc round-bottomed, long-necked flask in the cold with 100 cc of $H_2O$ and 5 cc of $H_3PO_4$ for several hours, or until the gum is completely swollen. Boil gently for 2 hours under a reflux condenser. A very small quantity of cellulose substance will remain undissolved.

While tragacanth yields a practically colorless soln when boiled with aqueous $H_3PO_4$, Indian gum, on the other hand, gives a pink or rose soln. This reaction may be used as a preliminary test for the detection of Indian gum.

Distil the hydrolyzed product with steam, using a spray trap to connect the distillation flask with the condenser. Continue the distillation until the distillate amounts to 600 cc, and the acid residue to about 20 cc. To avoid scorching of residue do not permit concentration of contents of distilling flask to less than 20 cc. Titrate the distillate with 0.1 *N* NaOH soln, using 10 drops of phenolphthalein indicator [**II, 8** (d)]. Correct the result by a blank determination and express as "volatile acidity" —the number of cc of 0.1 *N* NaOH soln required to neutralize the volatile (acetic) acid obtained by distilling with steam the products of the action of boiling aqueous $H_3PO_4$ on 1 g of the gum.

### 115 ETHYL ALCOHOL IN THE PRESENCE OF METHYL ALCOHOL[49]—TENTATIVE

(When methyl alcohol is present in small amounts, 5% or less)

#### PREPARATION OF STOCK SOLUTION OF METHYL ALCOHOL

*Solution A.*—Prepare a soln of methyl alcohol, adjusting its strength to 25% by volume (±0.1%).

*Solution B.*—Prepare a standard soln of methyl alcohol by making 20 cc of Solution A and 95 cc of absolute alcohol (or the equivalent of this amount in dilute alcohol) up to a volume of 2 liters. Make all transfers and dilutions at 20°.

### 116 DETERMINATION

*Total alcohols.*—Measure at room temp. (20°) 25 cc of sample, add 90 cc of $H_2O$, neutralize to litmus with dilute NaOH, distil, and dilute the volume of distillate to 100 cc at the same temp. as noted when the original aliquot was measured. Determine the total alcohol (as ethyl alcohol) from the specific gravity of the distillate in the usual way and estimate the percentage of alcohol in the original soln by means of the proper dilution factor. Test a portion of this distillate by the regular U. S. P. test for methyl alcohol, taking precaution to determine that HCHO, as such, is not present. If methyl alcohol is present, transfer 10 cc of the distillate to a separator, add 40 cc of saturated salt soln, shake with 25 cc of petroleum ether, and draw off the aqueous salt soln into a distilling flask. Wash the petroleum ether in the separator with two 10 cc portions of saturated salt soln, adding these to the portion already in the distilling flask. Distil, receiving the distillate in a 50 cc graduated flask. Calculate the quantity of ethyl alcohol to add to this distillate to make a 5% soln of total alcohol (assuming it to be all ethyl alcohol) when made up to 50 cc, add this calculated amount, and make up to a volume of 50 cc. Transfer 5 cc of this distillate to a 200 cc flask for color comparison with standards.

*Color standards.*—Transfer to 200 cc flasks a series of aliquots, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 cc of Solution B, adding 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5, and 0 cc, respectively, of 5% ethyl alcohol. (These amounts of methyl alcohol represent percentages in the original unknown soln when the unknown is diluted as outlined under **116**.

*Methyl alcohol* (substantially as given in the U. S. P.).—To each of the standards and to the unknown, add 1 cc of $H_3PO_4$ (1+1) and 2 cc of 3% $KMnO_4$ soln and allow the mixtures to stand for 10 min. Add 1 cc of 10% oxalic acid soln and allow the mixtures to stand until they have become clear or transparent. Add 5 cc of $H_2SO_4$ soln (1+3) and 5 cc of freshly prepared fuchsin-sulfurous acid mixture* and allow the soln to stand for 1½ hours. Dilute to 200 cc, mix thoroly, and transfer equal quantities to a series of test tubes of uniform color and diameter for color comparison. Compare the unknown with the standard which it most nearly approaches in color intensity, approximating intervals less than 0.5%, if desired. The value obtained represents the percentage of methyl alcohol in the original sample.

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* Dissolve 0.2 g of fuchsin in 120 cc of hot $H_2O$ and 2 g of $Na_2SO_3$ in 20 cc of $H_2O$. Mix, add 2 cc of HCl, and dilute to 200 cc.

*Ethyl alcohol.*—To obtain the percentage of ethyl alcohol, deduct the percentage of methyl alcohol, as determined colorimetrically, from the percentage of total alcohols, as previously determined.

## ARSENICALS

### ARSENIC IN IRON-ARSENIC TABLETS[50]—TENTATIVE

**117** REAGENTS

**(a)** *Nitric acid.*—Fuming.

**(b)** *Sulfuric acid.*

**(c)** *Hydrochloric acid.*

**(d)** *Ammonium oxalate.*—Saturated soln.

**(e)** *Sodium chloride.*

**(f)** *Sodium bromide.*

**(g)** *Ferrous sulfate or hydrazine sulfate.*

**(h)** *Methyl orange indicator.*—Dissolve 1 g of methyl orange in $H_2O$ and dilute to 1 liter.

**(i)** *Standard soln of potassium bromate (or of iodine).*—Standardize against pure $As_2O_3$. (The strength of this soln is a matter of choice. 0.5626 g of $KBrO_3$ dissolved in $H_2O$ and diluted to 1 liter will give a soln that is 0.02021 *N*, 1 cc of which = 1 mg of $As_2O_3$.)

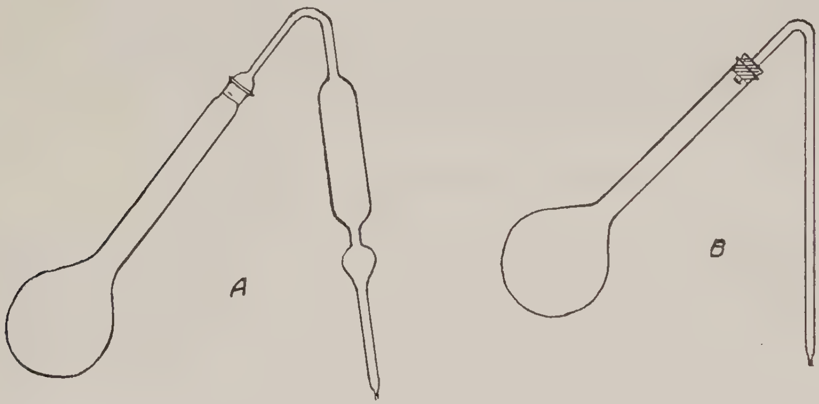

FIG. 38.—APPARATUS FOR THE DETERMINATION OF ARSENIC IN IRON-ARSENIC TABLETS

APPARATUS

Use either the Ramberg-Sjöström arsenic flask, which consists of a 300 cc Kjeldahl flask provided with a specially shaped outlet tube connected with the flask by means of a ground joint (*A*, Fig. 38), or a 300 cc Kjeldahl flask provided with an outlet tube, the internal diameter of the main part of which is about 13 mm and that of the contracted tip about 5 mm, connected with the flask by means of a rubber stopper (*B*, Fig. 38).

### 118 DETERMINATION

Weigh and place in the flask 5–10 tablets or pills, add 10–15 cc of $H_2O$, and allow to soak for ½ hour. Then add, in small portions at a time, 20 cc of fuming $HNO_3$, cooling if necessary to prevent loss by frothing. When the reaction has ceased, add carefully and in small portions at a time 25–28 cc of $H_2SO_4$. Place the flask in an inclined position on an asbestos mat and heat over a small flame. As soon as the greater part of the $HNO_3$ has been driven off and while still heating, drop in 8 cc of fuming $HNO_3$ through a suitably placed separator and heat over a larger flame until $SO_3$ is evolved. If, after cooling, the $H_2SO_4$ above the precipitated sulfates is not colorless or pale yellow and the precipitated sulfates are not free from gray or black particles, heat the contents of the flask further with an additional 10 cc of fuming $HNO_3$. (It is essential that all organic matter be destroyed.) To the cooled mixture add 30 cc of the saturated soln of $NH_4$ oxalate; heat until fumes of $SO_3$ are evolved and, to insure complete destruction of the oxalic acid, for 10 min. thereafter over a low flame; cool; and add while gently whirling the flask 20 cc of $H_2O$. Dry the neck of the flask over a small flame and add 30 g of NaCl, 5 g of $FeSO_4$ (or 1 g of hydrazine sulfate), 1 g of NaBr, and 25 cc of HCl. Mix the contents of the flask and connect the delivery tube. If the Ramberg-Sjöström apparatus is used, moisten the ground-glass joint with a drop of $H_2SO_4$. Fix the flask in an inclined position with the tip of the outlet tube about 1 cm under the surface of 150 cc of $H_2O$ in an Erlenmeyer flask surrounded by ice or by cold $H_2O$. Distil at such a rate that the bend at the top of the tube becomes warm in 4 min. and the lower end in about 8 min. from the time the heat is applied. Discontinue the distillation at the end of 10 min., but before removing the flame lift the distillation flask until the tip of the outlet tube is above the $H_2O$ in the receiving flask. Let the outlet tube drain, remove the receiver, and either titrate with the standard soln of $KBrO_3$, using 2 drops of methyl orange indicator (the red color of the indicator at the end point may fade slowly, but the color should persist for at least 1 min. upon addition of another drop of the indicator), or nearly neutralize with NaOH, add 4–5 g of $NaHCO_3$, and titrate with the standard soln of I, using starch indicator.

### 119 ARSENIC IN SODIUM CACODYLATE[51]—OFFICIAL

Transfer 0.2 g of the sample, accurately weighed, to a Kjeldahl flask. Add 10 g of $K_2SO_4$, 0.3 g of starch, and 20 cc of $H_2SO_4$. Digest over a low flame until frothing has ceased. Continue the digestion 4 hours or until the mixture is colorless. Cool, dilute with $H_2O$, and transfer to a 500 cc Erlenmeyer flask. Add NaOH soln (1+1) slowly until alkaline to litmus paper and acidify with $H_2SO_4$. Place the flask in $H_2O$ until thoroly cooled, add 5 g of $NaHCO_3$, and titrate with 0.1 *N* I soln. Conduct a blank using the same quantities of reagents. 1 cc of 0.1 *N* I soln = 0.00375 g of As, or to 0.008 g of anhydrous Na-cacodylate.

### 120 ARSENIC IN IRON METHYLARSENATES[52]—OFFICIAL, FIRST ACTION

Transfer a suitable quantity of the sample (0.2 g, if practicable) to a Kjeldahl flask. Add 10 g of $K_2SO_4$, 0.3 g of starch, and 20 cc of $H_2SO_4$. Digest over a low heat until frothing has ceased and continue the digestion over a slightly higher flame until the mixture is colorless. Cool, and add 20 cc of $H_2O$. Dry the neck of the flask over a small flame; cool the contents; and add 30 g of NaCl, 5 g of $FeSO_4$, 1 g of NaBr, and 25 cc of HCl. Then distil as directed in the method for the determination of As in iron-arsenic tablets. Conduct a blank using the same quantities of reagents.

## ARSENIC IN ARSPHENAMINE AND NEOARSPHENAMINE[53]—OFFICIAL

121

### REAGENTS

(a) *Hydrogen peroxide soln.*—3%.
(b) *Oxalic acid soln.*—Dissolve 1 g in 100 cc of $H_2O$.
(c) *Potassium iodide.*
(d) *Potassium permanganate.*—Finely ground.
(e) *Potassium permanganate soln.*—Dissolve 1 g in 100 cc of $H_2O$.
(f) *Sodium thiosulfate soln.*—0.1 *N*.
(g) *Sulfuric acid soln.*—10% by volume.
(h) *Sodium sulfite soln.*—Dissolve 2 g in 100 cc of $H_2O$.
(i) *Sodium hydroxide soln.*—(1+1).
(j) *Sodium bicarbonate.*
(k) *Iodine soln.*—0.1 *N*.

122

### DETERMINATION

Mix 0.2 g sample with 5 cc of 10% $H_2SO_4$ in a 500 cc Erlenmeyer flask fitted with a ground-glass stopper. Add 1 g of finely powdered $KMnO_4$ in small portions, mix thoroly, and allow to stand for 10 min. Add 10 cc of $H_2SO_4$ in 2 cc portions. Shake thoroly after each addition. Allow to digest 10 min., rotating the flask frequently during this period. Add 5 cc of $H_2O_2$ soln, and continue adding this soln dropwise until the brown precipitate disappears. To remove excess of peroxide, add 25 cc of $H_2O$, boil gently for 10 min., and carefully add a few drops of a 1% soln of $KMnO_4$ until the pink color is just permanent. To remove excess of permanganate, add a drop or two of oxalic acid soln. Dilute with 50 cc of $H_2O$. When the soln is cool, add 2 g of KI, stopper flask tightly, and let stand for 1 hour in a cool place.

Add from a buret, avoiding excess, the $Na_2SO_3$ soln to decolorize the liberated I. Add NaOH soln until slightly alkaline to litmus paper and render slightly acid with HCl. Place the flask in cold $H_2O$ until the soln is thoroly cooled; add 5 g of $NaHCO_3$, and titrate with 0.1 *N* I soln. Conduct a blank, using the reagents under the same conditions, and deduct the amount of 0.1 *N* sodium thiosulfate consumed. 1 cc of 0.1 *N* I soln = 0.00375 g of As.

## CHLOROFORM AND CARBON TETRACHLORIDE[54]—TENTATIVE

123

### REAGENTS

(a) *Alcoholic potassium hydroxide.*—Dissolve 30 g of KOH in 30 cc of $H_2O$. Cool, and dilute to 100 cc with methyl alcohol.

(b) *Alcoholic potassium hydroxide.*—Dissolve 35 g of KOH in sufficient methyl alcohol to make 100 cc. (This is a saturated soln.)

(c) *Silver nitrate soln.*—0.1 *N*.

(d) *Ammonium or potassium thiocyanate soln.*—0.05 *N*. Adjust by titrating against 0.1 *N* $AgNO_3$ soln.

(e) *Nitric acid.*

(f) *Ferric ammonium sulfate indicator.*—Dissolve 8 g of ferric ammonium sulfate in sufficient $H_2O$ to make 100 cc.

124

### WEIGHING OF SAMPLE

(1) *Chloroform or Carbon Tetrachloride:*

Carefully transfer 30 cc of alcoholic KOH soln into an air-dried, 60–70 cc pressure bottle and stopper. Do not moisten the neck of the bottle with the reagent. [Use Reagent (a) for $CHCl_3$ and Reagent (b) for $CCl_4$ and for mixtures of $CHCl_3$ or $CCl_4$.] Weigh the stoppered bottle with the contents. (The weighing may be conveniently made by suspending the bottle on the balance by means of the clamp that holds the stopper.)

Immediately after opening the bottle, add about 1 cc of the sample from a 1 cc pipet, holding the pipet just above the top level of the reagent in the pressure bottle. As the level of the reagent rises with the draining of the sample into the bottle, raise the pipet correspondingly so as to avoid contact with the reagent. Avoid having the bottle open longer than necessary, 20 seconds being a convenient time. Stopper the bottle so as to assure a tight fit and weigh. Determine the weight by difference. Proceed as under **125.**

(2) *Carbon Tetrachloride in Capsules:*

Ascertain the gross weight of a representative number of capsules. Open the capsules and transfer the contents to a suitable flask. Weigh the dried empty capsules and determine the average net contents. Proceed as directed under (1), using the composite sample.

(3) *Chloroform or Carbon Tetrachloride Mixtures:*

Proceed as directed under (1), using not more than 10 cc of the mixture containing 0.08–1.6 g of $CHCl_3$ or $CCl_4$. Note the temp. of the mixtures. Ascertain the volume-equivalent of the weighed sample. Weigh a definite volume of the mixture at the same temp., using a 50 or 100 cc volumetric flask, and calculate.

NOTE.—If desired, the sample may be measured directly with a pipet instead of being weighed, or a measured volume may be diluted with methyl alcohol to some definite volume and thoroly mixed and a suitable aliquot of this dilution used for the determination.

### **125** DETERMINATION

If the sample is a mixture, mix the contents of the bottle by gentle swirling and allow the bottle to stand about an hour (30 min. is sufficient for $CHCl_3$, pure or nearly so). Then place the bottle in a wire basket and set the basket in a water bath at room temp. Invert a tin can over the bottle and cover with a towel to prevent injury to the analyst in case the bottle should burst. Heat the bath to boiling and maintain at this temp. for 1 hour (15 min. is sufficient for $CHCl_3$, pure or nearly so).

Cool gradually, transfer the contents of the pressure bottle to a 200 cc volumetric flask, and wash out the bottle thoroly with $H_2O$, draining the washings into the flask. Bring to room temp., fill to mark with $H_2O$, and mix.

Transfer a suitable aliquot to a 100 cc volumetric flask and acidify with $HNO_3$ adding about 2 cc in excess. Add 25 or 50 cc of 0.1 *N* $AgNO_3$ (an excess), shake thoroly, fill to mark with $H_2O$, and mix. Filter the mixture through a dry filter into a dry flask, rejecting the first 20 cc of the filtrate. To a 50 cc aliquot of the filtrate, add 3 cc of ferric ammonium sulfate indicator and titrate the excess 0.1 *N* $AgNO_3$, using 0.05 *N* $NH_4$- or K-thiocyanate.

If the original sample contains chloride, which is precipitated by $AgNO_3$, determine the quantity and make correction.

If the original sample contains sugar or other organic material and (after the saponification of the $CHCl_3$ or $CCl_4$ and dilution of the mixture with $H_2O$) is highly colored, thus interfering with the titration, transfer the contents of the pressure bottle to a Ni crucible with the aid of $H_2O$. Evaporate to dryness and char the residue. Allow to cool, treat with $H_2O$, and filter into a suitable volumetric flask, washing the residue and filter with $H_2O$ until free from chloride. Fill to mark with $H_2O$ and mix. Determine the chloride as directed in the preceding paragraph.

Make a blank test, using in the pressure bottle the same quantities of solvents and reagents as when the sample is present, and apply such correction as may be necessary. 1 cc of 0.1 *N* $AgNO_3 = 0.003979$ g of $CHCl_3$ or 0.003846 g of $CCl_4$.

### CHLOROFORM IN MIXTURES[55]—TENTATIVE

**126** REAGENTS

(**a**) *Alcoholic potassium hydroxide.*—Dissolve 30 g of KOH (free from chloride) in 30 cc of $H_2O$ and sufficient methyl alcohol to make 100 cc. Allow to stand several days and decant clear liquid.

(**b**) *Alcohol.*—95% ethyl alcohol.

(**c**) *Nitric acid.*—68%.

(**d**) *Silver nitrate.*—Dissolve 10 g of $AgNO_3$ in sufficient $H_2O$ to make 500 cc.

(**g**) *Phenolphthalein.*—Dissolve 0.1 g in sufficient 95% alcohol to make 100 cc.

**127** DETERMINATION

Place 1 g of $CaCO_3$ and 75 cc of alcohol in a 250 cc Kjeldahl distilling flask and carefully pipet into this mixture 20 cc of the preparation to be examined, being careful not to agitate the mixture and to keep the tip of the pipet just below the surface of the liquid. Connect the flask with a straight-bore condenser and distil into a previously cooled citrate bottle immersed in cracked ice and containing 25 cc of alcoholic KOH soln into which the tip of the delivery tube should extend.

When 70 cc of the alcohol has been distilled over (judged by marking the bottle previously), discontinue the distillation and wash the receiving tube with about 10 or 15 cc of distilled $H_2O$, collecting the washings in the citrate bottle. Stopper the bottle; gently agitate, taking care to prevent the soln from coming in contact with the rubber washer; and allow to stand overnight at room temp. Heat on a steam bath for 1 hour, remove from the bath and allow to cool. Empty the contents of the bottle into a 500 cc beaker and wash the bottle with distilled $H_2O$ until the washings are no longer alkaline to phenophthalein, adding each washing to the main soln. Now add 15 cc of $HNO_3$ and an excess of $AgNO_3$, stir well, and allow the mixture to stand in a dark place for 15 min. Collect the precipitate upon a Gooch crucible that has been previously prepared, dried at 105°, and weighed. Wash the precipitate with several portions of distilled water, then with 5 cc of alcohol followed by a 5 cc portion of ether. Dry at 105° and weigh.

Each gram of AgCl corresponds to 0.027765 g of $CHCl_3$. Assuming that 1 minim of $H_2O$ weighs 0.06161 g and that the specific gravity of $CHCl_3$ is 1.475 (average of the U. S. P. limits), then 1 minim of $CHCl_3$ weighs 0.090936 g, and basing calculations on 1 fluid ounce measuring 29.57 cc, the factor for grams of AgCl to minims of $CHCl_3$ per fluid ounce is 4.5142 for the 20 cc sample.

### MERCUROUS CHLORIDE (CALOMEL) IN TABLETS[56]—OFFICIAL, FIRST ACTION

**128** REAGENTS

(**a**) *Purified iodine.*—Prepare as directed under **3** (**a**).

(**b**) *Standard sodium thiosulfate soln.*—Prepare as directed under **3** (**b**).

(**c**) *Standard iodine soln.*—Dissolve about 14 g of I in a soln containing 18 g of KI in 100 cc of $H_2O$ and dilute to 1 liter. Standardize this soln against the standard $Na_2S_2O_3$ soln.

**129** DETERMINATION

Count and weigh a representative number of tablets. Pulverize a quantity of tablets and weigh accurately a sufficient portion of the well-mixed sample to represent 3–4 grains (0.19–0.26 g) of calomel. Transfer to a 200 cc glass-stoppered Erlenmeyer flask, add about 50 cc of $H_2O$, acidify with acetic acid, and after the soluble fillers have dissolved decant with the aid of suction thru a tightly packed asbestos mat placed on the removable perforated plate of a Caldwell crucible. Wash once with water by decantation, then successively with alcohol and ether. Transfer the removable plate holding the mat and insoluble material to the original flask, washing into the flask any insoluble material adhering to the sides of the crucible. Add 2.5 g of KI, 10 cc of $H_2O$, and then 30 cc of the standard I soln. Allow the mixture to stand, with frequent and fairly vigorous agitation, for about 1½ hours, or until soln of the calomel is complete. Titrate with the standard thiosulfate soln and add about 1 cc in excess. Then titrate back with the standard I soln, using starch indicator [**VI, 3 (e)**], until a permanent blue color is obtained. 1 cc of 0.1 *N* I soln = 0.02361 g of calomel.

### 130 CALOMEL IN CALOMEL OINTMENT[57]—TENTATIVE

Weigh accurately about 1 g of the ointment, transfer to a 250 cc glass-stoppered Erlenmeyer flask, and treat with about 50 cc of $CHCl_3$. When the base is dissolved, decant through a dry, closely packed asbestos mat in a Caldwell crucible, using light suction. Wash the flask and contents several times with 20 or 30 cc portions of $CHCl_3$, decanting thru the crucible. Allow any residual $CHCl_3$ in the flask to evaporate and transfer the asbestos mat and contents to the flask, wiping the sides of the crucible and the mouth of the flask with a damp piece of filter paper and adding it to the contents of the flask. Then add 2.5 g of KI and 50 cc of 0.1 *N* I soln, stopper, and mix the contents well. Allow the flask to stand about 1½ hours or until soln of the calomel is complete, agitating it frequently and fairly vigorously. Titrate with 0.1 *N* $Na_2S_2O_3$, adding 1 or 2 cc in excess and using starch as indicator. When all traces of I have disappeared, titrate back with the standard I soln until a blue color is obtained. 1 cc of 0.1 *N* I = 0.02361 g of calomel.

## MERCUROUS IODIDE[58]—TENTATIVE

### 131 REAGENTS

(**a**) *Purified iodine.*—Prepare as directed under **3** (**a**).
(**b**) *Standard sodium thiosulfate soln.*—Prepare as directed under **3** (**b**).
(**c**) *Standard iodine soln.*—Prepare as directed under **128** (**c**).

### 132 DETERMINATION

Count and weigh a representative number of tablets. Pulverize a quantity of tablets and weigh accurately a sufficient portion of the well-mixed sample to represent 3–4 grains (0.19–0.26 g) of HgI. Transfer the sample to a 200 cc glass-stoppered Erlenmeyer flask, add about 50 cc of $H_2O$, acidify with acetic acid, and after the soluble fillers have dissolved, decant with the aid of suction thru a tightly packed asbestos mat placed on the removable perforated plate of a Caldwell crucible. Wash once with $H_2O$ by decantation, then successively with alcohol and ether. Transfer the removable plate holding the mat and insoluble material to the original flask, washing into the flask any insoluble materials adhering to the sides of the crucible. Add 2.5 g of KI and 30 cc of the standard I soln. Allow the mixture to stand, with frequent and fairly vigorous agitation, for about 1½ hours, or until soln of the HgI is complete. Titrate with the standard thiosulfate soln and add 1 or 2 cc in excess. When all traces of I have disappeared, titrate back with standard I, using starch indicator. 1 cc of 0.1 *N* I soln = 0.03275 g of HgI.

NOTE.—Some commercial tablets are difficult to filter without loss of HgI thru the asbestos mat. A few drops of alumina cream, washed free from $NH_3$, placed on the mat before filtration is started, satisfactorily prevents loss tho it retards the filtration.

## SILVER PROTEINATES[59]

### 133 *Acidity or Alkalinity—Tentative*

Dialyze 1 g of the sample as directed under **135** and titrate a portion of the clear soln representing 0.5 g of the sample with either 0.02 *N* HCl or 0.02 *N* NaOH soln, as required, using phenolphthalein indicator. Calculate acidity as percentage of HCl and alkalinity as percentage of NaOH.

### 134 TOTAL SILVER[60]—OFFICIAL

Place 1 g, accurately weighed, in a 500 cc Kjeldahl flask; add 15 cc of concentrated $H_2SO_4$ and then 10 cc of concentrated $HNO_3$; place on a steam bath for a few min., with occasional rotation, to insure a homogeneous mixture; and boil to white fumes. Add more $HNO_3$, boil again to a clear colorless soln, and cool. Add 100 cc of distilled $H_2O$ and boil until free of nitrogen oxides. Cool, dilute to 300 cc, add 5 cc of $HNO_3$ and 5 cc of ferric ammonium sulfate soln and titrate with 0.1 *N* KCNS. 1 cc of 0.1 *N* KCNS = 0.010788 g of Ag.

### 135 DETECTION AND ESTIMATION OF IONIZABLE SILVER COMPOUNDS[61]—OFFICIAL

Weigh a strip of commercial dialyzing tubing 55 mm wide and about 1 foot long, wet with distilled $H_2O$ until uniformly pliable, shake free of adhering $H_2O$, and partially dry by rolling in a clean paper towel. Reweigh while still moist and place in a 250 cc beaker. (Sheets of dialyzing parchment paper may be used in place of tubing.) Over one end of a glass tube 10 cm long and approximately 2.5 cm in diameter, fold and secure by means of a rubber band a square piece of parchment paper in the form of a sack of sufficient size to hold the sample soln. Dialyzing material should be kept in a humid container to prevent breaking when handled.) Weigh 1 g of the sample, dissolve in 15 cc of $H_2O$, and transfer to the dialyzing tube. Calculate, and add sufficient $H_2O$ to the beaker to make a total of 100 cc. (This insures 20 cc in the dialyzing tube and 80 cc in the beaker.) Adjust the tubing to form a "U" in the beaker, cover with a watch-glass, and place in a cool dark closet for 24 hours.

## IODOFORM[62]—TENTATIVE

### 136 REAGENTS

(a) *Silver nitrate soln.*—0.1 *N*.

(b) *Ammonium thiocyanate soln.*—0.05 *N*. Standardize against 0.1 *N* $AgNO_3$ soln using an equal volume of alcohol and 3 cc of ferric ammonium sulfate soln as indicator.

(c) *Nitric acid.*—68%.

(d) *Ferric ammonium sulfate indicator.*—Dissolve 8 g of $Fe(NH_4)(SO_4)_2 . 12H_2O$ in 100 cc of $H_2O$.

(e) *Alcohol.*—95% by volume.

### 137 DETERMINATION

Weigh accurately about 0.25 g of $CHI_3$ and transfer quantitatively to a 200 cc Erlenmeyer flask. Add 40 cc of alcohol, swirl gently until the $CHI_3$ is dissolved, filter if necessary, and immediately add 40 cc of 0.1 *N* $AgNO_3$ and 10 cc of $HNO_3$. Swirl gently for about 5 min., allow to stand at room temp. for 2–3 hours, and then swirl occasionally as an aid in flocculating the AgI. Titrate the excess 0.1 *N* $AgNO_3$ with 0.05 *N* $NH_4CNS$, using 3 cc of ferric ammonium sulfate as an indicator. 1 cc of 0.1 *N* $AgNO_3$ = 0.01313 g of $CHI_3$. Or, filter, collecting the AgI on a dried and accurately weighed Gooch crucible, wash with $H_2O$ and finally with alcohol, and dry to constant weight at about 125°. 1 g of AgI = 0.5590 g of $CHI_3$.

### BARBITAL AND PHENOBARBITAL[63]—OFFICIAL

138 REAGENTS

(a) *Alkaline salt soln.*—Dissolve 20 g of NaOH in $H_2O$, dilute to 1 liter, add NaCl to saturation, and filter.

(b) *Solvent.*—Mix 20 cc of alcohol, 10 cc of ether, and 70 cc of $CHCl_3$.

139 PREPARATION OF SAMPLE

If in tablets, ascertain the average weight and powder a representative number.

140 DETERMINATION

Transfer 0.3 g of the powdered sample to a separator. Dissolve in 10 cc of the alkaline salt soln. If tablet lubricants are present, wash with 15 cc of absolute ether and decant from top of separator into a small beaker. Add 2 cc of strong HCl to the alkaline soln, then 5 cc of $H_2O$ to prevent supersaturation of salt. Extract 5 times, using 30, 20, 20, 10, and 10 cc portions of the solvent. Combine the solvent in a second separator and wash with 2 cc of $H_2O$ acidified with a drop of HCl. Filter the solvent thru a pledget of cotton into a small weighed beaker. Evaporate on a steam bath with the aid of an electric fan, heat for 10 min. at 90–100°, cool in a desiccator, and weigh. Test for complete extraction with 10 cc of solvent and evaporate in a separate beaker.

141 *Melting Point—Tentative*

Use the U. S. P. method.[64]

142 CHAULMOOGRA OIL

*(a) Preparation of Sample*[65]*—Tentative*

If the sample is solid or contains solid particles, warm it until the solids have melted. Shake the entire sample until thoroly mixed and allow it to cool to room temp.

143 *(b) Solubility in Alcohol—Tentative*

To 25 cc of the oil in a suitable graduated apparatus (a Rose and Hertzfeld fusel oil apparatus is very satisfactory), add 100 cc of alcohol and shake thoroly for 15 min. Allow the mixture to stand overnight and observe the reading of the lower layer. Calculate the percentage by volume of oil dissolved by the alcohol.

144 *(c) Examination of Alcohol-Soluble Portion—Tentative*

Transfer the mixture obtained above to a separator, drain off the oily layer, and evaporate the alcoholic soln on a steam bath to constant weight. Determine the acid number and the I number of the residue (U. S. P. X, 1926, pp. 427, 445).

145 *(d) Viscosity—Tentative*

Determine viscosity as directed in U. S. P. X, p. 467, preferably by the Engler or Saybolt apparatus.

### OIL OF CHENOPODIUM[66]—TENTATIVE

146 REAGENTS

(a) *Standard ferric ammonium sulfate soln.*—Dissolve 39.214 g of pure, crystallized ferrous ammonium sulfate $[Fe\ (NH_4)_2(SO_4)_2.6H_2O]$ in 200 cc of $H_2O$ in a liter flask, add 30 cc of $H_2SO_4$, and mix well. Weigh exactly 3.16 g of $KMnO_4$, dissolve the salt in 200 cc of warm $H_2O$, and slowly add to the soln in the flask, with stirring. The permanganate soln should be just sufficient to oxidize the iron salt, but it is well to add the last few cc in small portions. Cool the soln and dilute it to 1 liter with $H_2O$.

(b) *Standard titanium trichloride soln.*—Add 100 cc of commercial 15–20% $TiCl_3$ soln to 200 cc of HCl, boil for 1 min., cool, and dilute to 4500 cc with $H_2O$. Place the soln in a container with H atmosphere provision and allow to stand for 2 days for absorption of residual O. Preserve the $TiCl_3$ soln in an atmosphere of H (see **XXI,** Fig. 20), taking care to have all the joints air-tight, and covering the stoppers (preferably countersunk) with suitable wax. Standardize the soln by titrating 20 cc of Reagent (a) against the $TiCl_3$ soln in a protective stream of $CO_2$, using 1 cc of 5% KCNS soln as an indicator. 1 cc of 0.1 $N$ $Fe_2(SO_4)_3 = 0.01545$ g of $TiCl_3$.

### 147 PREPARATION OF SAMPLE

Weigh 1 cc of the oil in a 100 cc volumetric flask and dilute to volume with 95% alcohol.

### 148 DETERMINATION

Fit an Erlenmeyer flask with a Bunsen valve thru which a current of $CO_2$ can be passed continuously, and place 50 cc of Reagent (b) in the flask. Add 10 cc of the diluted soln of the oil, close the flask with the Bunsen valve, and heat the contents almost to boiling for 2 min. (Prolonged heating has no effect if the contents are not boiled vigorously.) If the pale violet color of the $TiCl_3$ disappears, add more of Reagent (b) to insure an excess. (The formation of a white precipitate of titanic oxide does not interfere with the determination.) Add 1 cc of a 5% soln of KCNS and titrate back the excess of $TiCl_3$ with Reagent (a) in a $CO_2$ atmosphere until a faint, permanent, brownish red color is obtained.

### 149 CALCULATION

Subtract the quantity of ferric ammonium sulfate used, expressed in equivalent mg of $TiCl_3$, from the number of mg of $TiCl_3$ taken. The difference is the number of mg of $TiCl_3$ oxidized by the oil taken. Convert the mg of $TiCl_3$ oxidized into ascaridole by dividing by the factor 1.284 (1 g of ascaridole is reduced by 1.284 g of $TiCl_3$).

EXAMPLE: 0.9600 g of oil was made up to 100 cc and a 10 cc aliquot was heated with 50 cc of Reagent (b), (1 cc containing 0.0034 g of the salt). It then required 5.9 cc of Reagent (a), each cc equivalent to 0.01545 g of $TiCl_3$, to titrate back the soln. The grams of titanium trichloride oxidized is numerically equal to $(50 \times 0.0034) - (5.9 \times 0.01545)$, or 0.07885. The weight of oil in the aliquot was 0.0960 g. Hence the percentage of ascaridole $= \frac{0.07885 \times 100}{0.096 \times 1.284} = 72.1\%$.

## BIOSSAY OF DRUGS

### MYDRIATICS AND MYOTICS

*Cat-eye Method[67]—Official, first action*

### 150 APPARATUS

(a) *Mohr pipets.*—1 cc, graduated in 0.1 cc, with slender tips that deliver exactly 0.05 cc per drop.

(b) *N-filled electric lamps.*—100 watt or equally intense illumination.

### 151 ANIMALS

*Adult cats.*—In good physical condition, weighing over 1500 g, and accustomed to being handled.

### 152 PREPARATION OF SAMPLE

Dissolve, in approximately neutral distilled $H_2O$, a representative number of tablets, or a sufficient quantity of powder, to make a soln containing 1 mg of the alkaloid per cc of soln. If the alkaloids themselves are taken, add the equivalent quantities of acid to convert them into the corresponding salts. Add 2 drops of approximately 0.02 *N* acid per 50 cc of soln.

For great accuracy, the results of chemical assay upon the sample should be followed in the preparation of solns; when such accuracy is unnecessary, the declaration of strength on the label may be accepted as the basis for the preparation of the soln.

One drop of the respective concentrations of the following drugs has been found to be the minimum effective dose:

| MYDRIATICS | *mg per liter* |
|---|---|
| Atropine | 12 |
| Hyoscyamine | 4 |
| Scopolamine | 0.4 |
| Homatropine | 200 |
| Cocaine | 60 |
| Euphthalmin | 50,000 |
| Ephedrine (alkaloid) | 2,500 |
| Ephedrine salt (or synthetic) | 50,000 |
| Pseudoephedrine (alkaloid) | 2,500 |
| Pseudoephedrine (salt) | 80,000 |
| MYOTICS | |
| Pilocarpine | 25,000 |
| Physostigmine (eserine) | 10 |
| Arecoline | 10,000 |

### 153 DETERMINATION OF CAT'S THRESHOLD

Place a cat about 1 foot from a 100-watt electric lamp, and determine the maximum contractility of its pupils under this condition. Drop 0.05 cc of the freshly prepared standard mydriatic soln, obtained by diluting the 1 mg-per-cc soln, into the outer margin of one eye, leaving the other eye untreated as a control. Compress the inner canthus, while opening and closing the lids, until the fluid has apparently disappeared (10–30 seconds). Return cat to cage.

One and two hours after application (for atropine, 3 and 4 hours also), place cat under the same conditions, and note any differences in diameter between the pupils of the treated and the untreated eyes. (A satisfactory reaction is produced when the pupil of the treated eye is just perceptibly wider (0.5–1.0 mm) than the pupil of the untreated eye.) Do not use the same eye for another assay for at least 24 hours.

If the concentrations given fail to produce a satisfactory reaction, repeat the test with a stronger or weaker soln until the minimum effective concentration is found. (This concentration may vary somewhat for different cats, but it is essentially constant for the same cat.)

### 154 BIOASSAY OF UNKNOWN SOLUTIONS

Dilute the 1 mg-per-cc soln to be tested to the minimum effective concentration for the cats to be used, and drop 0.05 cc of this dilution into one eye of the cat, following the same procedure as in the determination of the minimum effective concentration. Also prepare stronger and weaker solns and apply to one eye of each of the other cats used. Test various concentrations until one is obtained that produces satisfactory mydriasis of the same degree as the standard soln when tested on two or more cats.

To obtain the mg of alkaloid present in each cc of the original soln, multiply the mg per cc found to be the cat's minimum effective concentration by the dilution employed. Knowing that the original soln was made to contain 1 mg of alkaloid per cc, calculate the quantity of mydriatic present, and express as percentage of the total alkaloid.

## ASSAY OF ERGOT[68] TENTATIVE

### 155 REAGENTS

(a) *Purified petroleum benzin.*—U. S. P. standard.

(b) *Menstruum I.*—Mix 20 cc of HCl with 490 cc of alcohol and 490 cc of $H_2O$.

(c) *Adrenalin hydrochloride soln.*

(d) *Menstruum II.*—Diluted alcohol U. S. P.

(e) *Locke-Ringer soln modified.*—Omit the use of $MgCl_2$ in the Locke-Ringer soln U. S. P.

### 156 APPARATUS

Use the isolated organ bath described in the U. S. P. X. under Liquor Pituitarii, modifying it by having a second glass container inside the warming bath. Two levers must be fixed, one above each glass container, to record side by side on the same drum. As a sixfold magnification of the movements is required, the levers should be longer than in the U. S. P. X. Method. Each lever should be supported at its midpoint. The arrangements for filling the containers with the Locke-Ringer soln may be those described in the U. S. P. assay for Liquor Pituitarii, and the same pattern of recording drum may be used.

### 157 PREPARATION OF SAMPLE

Grind the ergot to a No. 20 powder, pack the recently ground drug in a cylindrical percolator, and slowly percolate with purified petroleum benzin until a few drops of the percolate leave no greasy stain when evaporated from filter paper. Reject the benzin soln, remove the drug from the percolator, and dry it by exposure to the air.

Moisten the defatted drug with a 5% soln of $NaHCO_3$ and allow it to stand in a cold room for from 2 to 4 hours. Pack in a percolator, add more bicarbonate soln and allow to macerate from 16 to 24 hours in a cold room. Percolate slowly with $H_2O$ until the percolate is found by physiological tests to be practically free from amines. Allow to drain completely, remove all but the lowest inch of marc from the percolator, and remove $H_2O$ from it by strong expression or by centrifuging. Moisten the drug with a small quantity of Menstruum I. Repack the drug in the percolator and macerate overnight in a cold room. Add the remainder of Menstruum I, and when this has just disappeared from the surface gradually add Menstruum II, constantly maintaining a stratum of liquid above the drug. When the liquid begins to drop from the percolator, close the lower orifice, and, having closely covered the percolator, macerate for 48 hours (in a cold room), and then allow the percolation to proceed slowly, gradually adding Menstruum II until the drug is exhausted. Reserve the first 850 cc of the percolate, recover the alcohol from the remainder of the percolate, and concentrate the residue to a soft extract at a temp. not exceeding 60° (preferably in a vacuum distillation apparatus). Dissolve the extract in the reserved portion, mix thoroly, and assay a portion by the method given below. From the results thus obtained adjust the volume of the finished fluid extract by the addition of Menstruum II to make it conform to the required biological standard.

### 158 THE TEST

Use nonpregnant female rabbits weighing 2 kg or more, and which are at least 3 weeks past parturition. Kill the animal by a blow on the head, cut its throat, and then suspend by the hind legs until hemorrhage ceases. Remove the uterus. Cut a piece about 1 cm in length from one uterine horn. The remainder of the uterine horns may be placed between pieces of cotton wool dampened with Ringer's soln and kept, properly covered, at a temp. of 40–50°F for any subsequent tests within the next 10 days. Place the piece of uterine horn on the convex surface of a watch-glass and cut it open along the line of mesenteric attachment. Unfurl, and cut away the sides so that a piece 8–10 mm wide and farthest from the mesentery remains. Divide this piece longitudinally into two equal parts and suspend each in one of the glass containers of the bath holding the Locke-Ringer soln. Weight the recording levers so as to induce relaxation of the muscle. To each bath add 0.02 mg of adrenaline (as the hydrochloride in soln). If necessary, increase simultaneously the dose in each bath by increments of 0.01 or 0.02 mg until a contraction which is maintained 2–3 min. is produced. Adjust the weights on the levers so that the extent of contraction in each bath is similar and conspicuously greater than any spontaneous contractions. Spontaneous contractions increase in amplitude as the muscle remains in the bath so that weighting should as a rule be greater than at first seems necessary. At a noted time add to one bath a dose of a specific alkaloid. (This may be 0.4 cc of a soln of ergotamine tartrate or ergotoxine phosphate, 1 in 30,000. The concentration may vary from 1 part in 10,000 to 1 part in 200,000, depending upon the response obtained in the muscle.) Thirty seconds later add to the other bath 0.4 cc of the unknown soln diluted approximately 30 times; 10 min. from the time of the additions to each bath add adrenalin to each bath in the dosage found to give a previously satisfactory contraction. Note the contraction produced on each muscle. If the contraction has been reduced to the same extent in both baths the concentration of the specific alkaloid in each bath is the same. If the contractions are not the same, repeat the procedure, using fresh uterine strips, the same dose of adrenaline and larger or smaller doses of the ergot preparation as indicated by the previous test.

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# XL. BACTERIOLOGICAL METHODS*

# XLI. MICROCHEMICAL METHODS*

* See note at bottom of p. xv.

# APPENDIX

## DEFINITIONS OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

For many years officials charged with the enforcement of state fertilizer control laws and members of the fertilizer industry in general have felt the need of some authoritative clearing house for fertilizer definitions and interpretations. Well-ordered fertilizer control is dependent upon such definitions and interpretations to meet emergencies or needs as they become apparent. After an open discussion of the question at the 37th Annual Convention of the Association of Official Agricultural Chemists, held in October 1921, a committee was appointed to be known as the Committee on Definitions of Terms and Interpretation of Results on Fertilizers. Five official chemists were selected from the states of Indiana, Massachusetts, Pennsylvania, South Carolina and Texas, an attempt being made to represent a geographically wide area of active control service work.

At the 38th Annual Convention, held in November 1922, the committee offered amendments to the by-laws of the Association to cover its proposed activities. The following year the committee held an open meeting and invited both control and commercial interests to propose and discuss subjects for consideration and action, a procedure that has been followed since that time. Tentative definitions and interpretations on five subjects were recommended. In 1925 these first five recommendations of the committee were made official by action of the Association, and at each subsequent annual convention some definitions and interpretations proposed by the committee have been made official. The association has now (1930) placed its official approval upon forty definitions proposed by this committee. Twenty-four other definitions and interpretations await the necessary action as to recommendation and publication in the Journal to become official.

At the 44th Annual Convention (1928) the Association voted to publish in the 1930 revision of *Methods of Analysis* the definitions and interpretations that have been made official. At the meeting held in 1930 the name of the committee was changed, to include the work on liming materials, to "Committee on Definitions of Terms and Interpretation of Results on Fertilizers and Liming Materials."

To facilitate reference the definitions are listed alphabetically in one group, and the interpretations are given in like manner in a separate group.

## DEFINITIONS

### ACIDULATED FISH TANKAGE. ACIDULATED FISH SCRAP

*Acidulated fish tankage, acidulated fish scrap*, is the rendered product derived from fish and treated with sulfuric acid.

### ACTIVATED SEWAGE PRODUCTS

*Activated sewage products* are those made from sewage freed from grit and coarse solids and aerated after being inoculated with microorganisms. The resulting flocculated organic matter is withdrawn from the tanks, filtrated with or without the aid of coagulants, dried, ground, and screened.

### AMMONIATED SUPERPHOSPHATE

*Ammoniated superphosphate* is a product containing superphosphate and/or dissolved bone and nitrogenous compounds, but without the addition of potash.

## ANALYSIS

*Analysis*, as applied to fertilizers, designates the percentage composition of the product expressed in terms of nitrogen, phosphoric acid, and potash in their various forms.

## ASHES FROM LEACHED WOOD

*Ashes from leached wood* are unleached ashes resulting from the burning of wood that has been exposed to or digested in water or other liquid solvent, as in the extraction of dyes, so that a part of the plant food has been dissolved and removed.

## BASIC PHOSPHATE SLAG

*Basic phosphate slag* is a by-product in the manufacture of steel from phosphatic iron ores. The product shall be finely ground and shall contain no admixture of materials other than what results in the original process of manufacture. It shall contain not less than twelve per cent (12%) of total phosphoric acid ($P_2O_5$), not less than eighty per cent (80%) of which shall be soluble in two per cent (2%) citric acid soln according to the Wagner method of analysis. Any other phosphate slag not conforming to this definition shall be designated *low grade.*

## BRAND AND BRAND NAME

A *brand* is a term, design, or trade mark used in connection with one or several grades of fertilizers.

A *brand name* is a specific designation applied to an individual fertilizer.

## CRUDE, INERT, OR SLOW-ACTING NITROGENOUS MATERIALS

*Crude, inert, or slow-acting nitrogenous materials* are unprocessed organic substances relatively high in nitrogen but having a very low value as plant food and showing a low activity by both the alkaline and neutral permanganate methods (below fifty per cent (50%) and eighty per cent (80%) respectively).

## DISSOLVED BONE

*Dissolved bone is* ground bone or bone meal that has been treated with sulfuric acid.

## DRIED BLOOD

*Dried blood* is the collected blood of slaughtered animals, dried and ground and containing not less than twelve per cent (12%) of nitrogen in organic forms.

## DRIED, PULVERIZED, OR SHREDDED MANURES

*Dried, pulverized, or shredded manures* are what the name indicates, and not mixtures of manures and other materials.

## FERTILIZER GRADE

*Fertilizer grade* shall represent only the minimum guarantee of its plant food expressed in terms of nitrogen (not ammonia), available phosphoric acid, and water-soluble potash.

## FISH TANKAGE, FISH SCRAP, DRY GROUND FISH, FISH MEAL FERTILIZER GRADE

*Fish tankage, fish scrap, dry ground fish, fish meal fertilizer grade* is the dried ground product derived from rendered or unrendered fish.

## GARBAGE TANKAGE

*Garbage tankage* is the rendered, dried, and ground product derived from waste household food materials.

## GROUND RAW BONE

*Ground raw bone* is dried ground animal bones that have not been previously steamed under pressure.

## GROUND STEAMED BONE

*Ground steamed bone* is ground animal bones that have been previously steamed under pressure.

## HOOF AND HORN MEAL

*Hoof and horn meal* is processed, dried, and ground hoofs and horns.

## KAINIT

*Kainit* is a potash salt containing potassium and sodium chlorides and sometimes sulfate of magnesia with not less than twelve per cent (12%) of potash ($K_2O$).

## LEACHED WOOD ASHES

*Leached wood ashes* are ashes from burned unleached wood with part of their plant food removed by artificial means or by exposure to rains, snows, or other solvent.

## MANURE SALTS

*Manure salts* are potash salts containing high percentages of chloride and from twenty per cent (20%) to thirty per cent (30%) of potash ($K_2O$). The term *double manure salts* is discontinued.

## MURIATE OF POTASH (COMMERCIAL POTASSIUM CHLORIDE)

*Muriate of potash* is a potash salt containing not less than forty-eight per cent (48%) of potash ($K_2O$), chiefly as chloride.

## NITRATE OF POTASH (COMMERCIAL POTASSIUM NITRATE)

*Nitrate of potash* is a salt containing not less than twelve per cent (12%) of nitrogen and forty-four per cent (44%) of potash ($K_2O$).

## NITRATE OF SODA (COMMERCIAL SODIUM NITRATE)

*Nitrate of soda* is commercial sodium nitrate containing not less than fifteen per cent (15%) of nitrogen, chiefly as sodium nitrate.

## PRODUCTS SECURED BY HEATING CALCIUM PHOSPHATE WITH ALKALI SALTS CONTAINING POTASH

*Products secured by heating calcium phosphate with alkali salts containing potash* are non-acid phosphates with potash. They are *not* potassium phosphate.

## SULFATE OF POTASH (COMMERCIAL POTASSIUM SULFATE)

*Sulfate of potash* is a potash salt containing not less than forty-eight per cent (48%) of potash ($K_2O$), chiefly as sulfate, and not more than two and one-half per cent (2.5%) of chlorine.

## SULFATE OF POTASH-MAGNESIA

*Sulfate of potash-magnesia* is a potash salt containing not less than twenty-five per cent (25%) of potash ($K_2O$), nor less than twenty-five per cent (25%) of sulfate of magnesia, and not more than two and one-half per cent (2.5%) of chlorine.

## SUPERPHOSPHATE

*Superphosphate* is a a cured mixture of rock phosphate and sulfuric acid and/or phosphoric acid. The grade shall always be used as a prefix to the name. Example: 16% Superphosphate. The use of the term *Acid Phosphate* is discontinued.

### TANKAGE

*Tankage* (without qualification) is the rendered, dried, and ground by-product, largely meat and bone from animals (slaughtered or that have died otherwise).

### UNIT OF PLANT FOOD

A *unit of plant food* is twenty (20) pounds, or one per cent (1%) of a ton.

### UNLEACHED WOOD ASHES

*Unleached wood ashes* are ashes from burned unleached wood that have had no part of their plant food removed and that contain four per cent (4%) or more of water-soluble potash ($K_2O$).

## INTERPRETATIONS

### ACTIVITY OF WATER-INSOLUBLE NITROGEN IN FERTILIZERS

The alkaline and neutral permanganate methods distinguish between the better and the poorer sources of water-insoluble nitrogen; they do not show the percentage availability of the material. The available nitrogen of any product is measured only by carefully conducted vegetation experiments.

(a) The methods shall be used on mixed fertilizers containing water-insoluble nitrogen amounting to three-tenths of one per cent (0.3%) or more of the weight of the material. If a total nitrogen exceeds the minimum guarantee and is accompanied by a low activity of the insoluble nitrogen, the over-run shall be taken into consideration in determining the classification of the water-insoluble nitrogen.

(b) The water-insoluble nitrogen in mixed fertilizers showing an activity below fifty per cent (50%) by the alkaline method and also below eighty per cent (80%) by the neutral method shall be classed as inferior. This necessitates the use of both methods, also the provision as to over-run in (a), before classifying as inferior.

### AMOUNT OF CHLORINE PERMISSIBLE IN FERTILIZERS IN WHICH THE POTASH IS CLAIMED AS SULFATE

*Chlorine in mixed fertilizers in which the potash is claimed as sulfate* shall not exceed one-half of one per cent (0.5%) more than what is called for in the minimum potash content based on the definition for sulfate of potash formulated by the committee. Calculate as follows: 0.05 times the percentage of potash found plus 0.5.

### BRAND NAME TO INCLUDE ANALYSIS OR GRADE OF FERTILIZER

*The analysis or grade of a fertilizer* should be included with its brand name, and so used by the manufacturer on sacks and in printed literature and by the control official in his reports and publications.

### FERTILIZER FORMULA

A *fertilizer formula* shall express the quantity and grade of the crude stock materials used in making a fertilizer mixture. For example: 800 pounds of 16 per cent superphosphate, 800 pounds of tankage (7.40 nitrogen and 9.15 total phosphoric acid), and 400 pounds of sulfate of potash-magnesia (twenty-six per cent (26%), potash).

### FINELY GROUND AS APPLIED TO BASIC PHOSPHATE SLAG

*Finely ground* in the definition of basic phosphate slag shall refer to actual size of particles as determined by the use of standard sieves, as follows: seventy per cent (70%) or more shall pass a 100-, and ninety per cent (90%) or more shall pass a 50-mesh sieve.

### FORM OF NITROGEN IN CALCIUM CYANAMIDE

The nitrogen in calcium cyanamide shall be considered as being of organic nature.

### THE TERM LIME AS APPLIED TO FERTILIZERS

The term *lime* shall not be used in the registration, labelling, or guaranteeing of fertilizers or fertilizing materials, unless the lime is in a form to neutralize soil acidity, such as the oxide, hydroxide, or carbonate, or equivalent magnesia compounds.

### ORDER OF TERMS

The *order of terms* in mixed fertilizers shall be nitrogen first, phosphoric acid second, and potash third.

### NAME OF A FERTILIZER MATERIAL USED AS THE BRAND NAME OR PART OF THE BRAND NAME OF A MIXED FERTILIZER

When the name of a fertilizer material is used as part of the brand name of a mixed fertilizer, as for example blood, bone or fish, the nitrogen or phosphoric acid shall be derived from or supplied entirely by the material named. When the name of a fertilizer material is used as a brand or as part of a brand and the nitrogen and phosphoric acid is not supplied by the material named, the word *brand* shall follow the name of the material. Example: *Fish Brand Fertilizer*.

### STATEMENT OF GUARANTEE

The *statement of guarantee* of mixed fertilizers shall be given in whole numbers.

## XLII. REFERENCE TABLES

1 International Atomic Weights . . . . . 496
2 Degrees Brix, Specific Gravity, and Degrees Baumé of Sugar Solutions. 497
3 Temperature Corrections to Readings of Saccharometers (Standard at 20°C) . . . . . 503
4 Domke's Table—Apparent Specific Gravity of Sucrose Solutions at 20°C. 504
5 Densities of Solutions of Cane Sugar at 20° . . . . . 506
6 Schönrock's Table for Determining Water in Sugar Solutions by Means of Abbé Refractometer . . . . . 510
7 Stanek's Correction Table for Determining Water in Sugar Solutions by Means of the Abbé Refractometer When Readings are Made at other Temperatures than 20°C. . . . . . 512
8 Hübener's Table for Determining Percentages by Weight of Sucrose in Sugar Solutions from Readings of the Zeiss Immersion Refractometer. 513
9 Munson and Walker's Table for Calculating Dextrose, Invert Sugar, Lactose, and Maltose . . . . . 514
10 Herzfeld's Table for Determining Invert Sugar in Raw Sugars . . . . . 520
11 Meissl and Hiller's Factors for Determining Invert Sugar in Materials in which, of the Total Sugars Present, more than 1.5 per cent is Invert Sugar and less than 98.5 per cent is Sucrose . . . . . 521
12 Wein's Table for the Determination of Maltose . . . . . 522
13 Soxhlet and Wein's Table for the Determination of Lactose . . . . . 523
14 Allihn's Table for the Determination of Dextrose . . . . . 524
15 Factors for 10 cc Soxhlet's Solution to be used in connection with the Lane-Eynon General Volumetric Method . . . . . 526
16 Factors for 25 cc Soxhlet's Solution to be used in connection with the Lane-Eynon General Volumetric Method . . . . . 527
17 Quisumbing and Thomas Table for Calculating Dextrose, Levulose, Invert Sugar, Lactose, and Maltose . . . . . 528
18 Kröber's Table for the Determination of Pentoses and Pentosans . . . . . 529
19 Relation between Specific Gravity at 15.56/15.56° C. and Alcohol Content of Mixtures of Ethyl Alcohol and Water . . . . . 532
20 Relation between Specific Gravity at 20/20° C. and Alcohol Content of Mixtures of Ethyl Alcohol and Water . . . . . 534
21 Relation between Specific Gravity at 25/25°C and Alcohol Content of Mixtures of Ethyl Alcohol and Water . . . . . 536
22 Alcohol Table for Calculating the Percentages of Alcohol in Mixtures of Ethyl Alcohol and Water from their Immersion Refractometer Readings at 17.5–25°C. . . . . . 538
23 For Determining Added Water in Milk by Means of the Freezing-Point Depression (based on Winter's Table) . . . . . 551
24 Density of Carbon Dioxide (Parr) . . . . . 556
25 Correction Factors for the Gasometric Determination of Carbon Dioxide. 558
26 Various Strength Solutions of the Common Acids, Alkalies, and Alcohol . . . . . 563

# XLII. REFERENCE TABLES

1 *International atomic weights*[1]

1931

| | SYMBOL | ATOMIC NUMBER | ATOMIC WEIGHT | | SYMBOL | ATOMIC NUMBER | ATOMIC WEIGHT |
|---|---|---|---|---|---|---|---|
| Aluminum | Al | 13 | 26.97 | Molybdenum | Mo | 42 | 96.0 |
| Antimony | Sb | 51 | 121.76 | Neodymium | Nd | 60 | 144.27 |
| Argon | A | 18 | 39.944 | Neon | Ne | 10 | 20.183 |
| Arsenic | As | 33 | 74.93 | Nickel | Ni | 28 | 58.69 |
| Barium | Ba | 56 | 137.36 | Nitrogen | N | 7 | 14.008 |
| Beryllium | Be | 4 | 9.02 | Osmium | Os | 76 | 190.8 |
| Bismuth | Bi | 83 | 209.00 | Oxygen | O | 8 | 16.0000 |
| Boron | B | 5 | 10.82 | Palladium | Pd | 46 | 106.7 |
| Bromine | Br | 35 | 79.916 | Phosphorus | P | 15 | 31.02 |
| Cadmium | Cd | 48 | 112.41 | Platinum | Pt | 78 | 195.23 |
| Calcium | Ca | 20 | 40.08 | Potassium | K | 19 | 39.10 |
| Carbon | C | 6 | 12.00 | Praseodymium | Pr | 59 | 140.92 |
| Cerium | Ce | 58 | 140.13 | Radium | Ra | 88 | 225.97 |
| Cesium | Cs | 55 | 132.81 | Radon | Rn | 86 | 222 |
| Chlorine | Cl | 17 | 35.457 | Rhenium | Re | 75 | 186.31 |
| Chromium | Cr | 24 | 52.01 | Rhodium | Rh | 45 | 102.91 |
| Cobalt | Co | 27 | 58.94 | Rubidium | Rb | 37 | 85.44 |
| Columbium | Cb | 41 | 93.3 | Ruthenium | Ru | 44 | 101.7 |
| Copper | Cu | 29 | 63.57 | Samarium | Sm | 62 | 150.43 |
| Dysprosium | Dy | 66 | 162.46 | Scandium | Sc | 21 | 45.10 |
| Erbium | Er | 68 | 167.64 | Selenium | Se | 34 | 79.2 |
| Europium | Eu | 63 | 152.0 | Silicon | Si | 14 | 28.06 |
| Fluorine | F | 9 | 19.00 | Silver | Ag | 47 | 107.880 |
| Gadolinium | Gd | 64 | 157.3 | Sodium | Na | 11 | 22.997 |
| Gallium | Ga | 31 | 69.72 | Strontium | Sr | 38 | 87.63 |
| Germanium | Ge | 32 | 72.60 | Sulfur | S | 16 | 32.06 |
| Gold | Au | 79 | 197.2 | Tantalum | Ta | 73 | 181.4 |
| Hafnium | Hf | 72 | 178.6 | Tellurium | Te | 52 | 127.5 |
| Helium | He | 2 | 4.002 | Terbium | Tb | 65 | 159.2 |
| Holmium | Ho | 67 | 163.5 | Thallium | Tl | 81 | 204.39 |
| Hydrogen | H | 1 | 1.0078 | Thorium | Th | 90 | 232.12 |
| Indium | In | 49 | 114.8 | Thulium | Tm | 69 | 169.4 |
| Iodine | I | 53 | 126.932 | Tin | Sn | 50 | 118.70 |
| Iridium | Ir | 77 | 193.1 | Titanium | Ti | 22 | 47.90 |
| Iron | Fe | 26 | 55.84 | Tungsten | W | 74 | 184.0 |
| Krypton | Kr | 36 | 82.9 | Uranium | U | 92 | 238.14 |
| Lanthanum | La | 57 | 138.90 | Vanadium | V | 23 | 50.95 |
| Lead | Pb | 82 | 207.22 | Xenon | Xe | 54 | 130.2 |
| Lithium | Li | 3 | 6.940 | Ytterbium | Yb | 70 | 173.5 |
| Lutecium | Lu | 271 | 175.0 | Yttrium | Y | 39 | 88.92 |
| Magnesium | Mg | 1 | 24.32 | Zinc | Zn | 30 | 65.38 |
| Manganese | Mn | 25 | 54.93 | Zirconium | Zr | 40 | 91.22 |
| Mercury | Hg | 80 | 200.61 | | | | |

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[1] Taken from J. Am. Chem. Soc., May, 1931.

*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.*[1] 2

| DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | SPECIFIC GRAVITY AT 20/4°C. | SPECIFIC GRAVITY AT 20/20°C. | DEGREES BAUME (MODULUS 145) | DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | SPECIFIC GRAVITY AT 20/4°C. | SPECIFIC GRAVITY AT 20/20°C. | DEGREES BAUME (MODULUS 145) |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.99823 | 1.00000 | 0.00 | 9.0 | 1.03403 | 1.03586 | 5.02 |
| 0.2 | 0.99901 | 1.00078 | 0.11 | 9.2 | 1.03485 | 1.03668 | 5.13 |
| 0.4 | 0.99979 | 1.00155 | 0.22 | 9.4 | 1.03567 | 1.03750 | 5.24 |
| 0.6 | 1.00056 | 1.00233 | 0.34 | 9.6 | 1.03649 | 1.03833 | 5.35 |
| 0.8 | 1.00134 | 1.00311 | 0.45 | 9.8 | 1.03732 | 1.03915 | 5.46 |
| 1.0 | 1.00212 | 1.00389 | 0.56 | 10.0 | 1.03814 | 1.03998 | 5.57 |
| 1.2 | 1.00290 | 1.00467 | 0.67 | 10.2 | 1.03897 | 1.04081 | 5.68 |
| 1.4 | 1.00368 | 1.00545 | 0.79 | 10.4 | 1.03980 | 1.04164 | 5.80 |
| 1.6 | 1.00445 | 1.00623 | 0.90 | 10.6 | 1.04063 | 1.04247 | 5.91 |
| 1.8 | 1.00523 | 1.00701 | 1.01 | 10.8 | 1.04146 | 1.04330 | 6.02 |
| 2.0 | 1.00602 | 1.00779 | 1.12 | 11.0 | 1.04229 | 1.04413 | 6.13 |
| 2.2 | 1.00680 | 1.00858 | 1.23 | 11.2 | 1.04312 | 1.04497 | 6.24 |
| 2.4 | 1.00758 | 1.00936 | 1.34 | 11.4 | 1.04395 | 1.04580 | 6.35 |
| 2.6 | 1.00836 | 1.01015 | 1.46 | 11.6 | 1.04479 | 1.04664 | 6.46 |
| 2.8 | 1.00915 | 1.01093 | 1.57 | 11.8 | 1.04562 | 1.04747 | 6.57 |
| 3.0 | 1.00993 | 1.01172 | 1.68 | 12.0 | 1.04646 | 1.04831 | 6.68 |
| 3.2 | 1.01072 | 1.01251 | 1.79 | 12.2 | 1.04730 | 1.04915 | 6.79 |
| 3.4 | 1.01151 | 1.01330 | 1.90 | 12.4 | 1.04814 | 1.04999 | 6.90 |
| 3.6 | 1.01230 | 1.01409 | 2.02 | 12.6 | 1.04898 | 1.05084 | 7.02 |
| 3.8 | 1.01309 | 1.01488 | 2.13 | 12.8 | 1.04982 | 1.05168 | 7.13 |
| 4.0 | 1.01388 | 1.01567 | 2.24 | 13.0 | 1.05066 | 1.05252 | 7.24 |
| 4.2 | 1.01467 | 1.01647 | 2.35 | 13.2 | 1.05151 | 1.05337 | 7.35 |
| 4.4 | 1.01547 | 1.01726 | 2.46 | 13.4 | 1.05236 | 1.05422 | 7.46 |
| 4.6 | 1.01626 | 1.01806 | 2.57 | 13.6 | 1.05320 | 1.05506 | 7.57 |
| 4.8 | 1.01706 | 1.01886 | 2.68 | 13.8 | 1.05405 | 1.05591 | 7.68 |
| 5.0 | 1.01785 | 1.01965 | 2.79 | 14.0 | 1.05490 | 1.05677 | 7.79 |
| 5.2 | 1.01865 | 1.02045 | 2.91 | 14.2 | 1.05575 | 1.05762 | 7.90 |
| 5.4 | 1.01945 | 1.02125 | 3.02 | 14.4 | 1.05660 | 1.05847 | 8.01 |
| 5.6 | 1.02025 | 1.02206 | 3.13 | 14.6 | 1.05746 | 1.05933 | 8.12 |
| 5.8 | 1.02105 | 1.02286 | 3.24 | 14.8 | 1.05831 | 1.06018 | 8.23 |
| 6.0 | 1.02186 | 1.02366 | 3.35 | 15.0 | 1.05916 | 1.06104 | 8.34 |
| 6.2 | 1.02266 | 1.02447 | 3.46 | 15.2 | 1.06002 | 1.06190 | 8.45 |
| 6.4 | 1.02346 | 1.02527 | 3.57 | 15.4 | 1.06088 | 1.06276 | 8.56 |
| 6.6 | 1.02427 | 1.02608 | 3.69 | 15.6 | 1.06174 | 1.06362 | 8.67 |
| 6.8 | 1.02508 | 1.02689 | 3.80 | 15.8 | 1.06260 | 1.06448 | 8.78 |
| 7.0 | 1.02588 | 1.02770 | 3.91 | 16.0 | 1.06346 | 1.06534 | 8.89 |
| 7.2 | 1.02669 | 1.02851 | 4.02 | 16.2 | 1.06432 | 1.06621 | 9.00 |
| 7.4 | 1.02750 | 1.02932 | 4.13 | 16.4 | 1.06519 | 1.06707 | 9.11 |
| 7.6 | 1.02832 | 1.03013 | 4.24 | 16.6 | 1.06605 | 1.06794 | 9.22 |
| 7.8 | 1.02913 | 1.03095 | 4.35 | 16.8 | 1.06692 | 1.06881 | 9.33 |
| 8.0 | 1.02994 | 1.03176 | 4.46 | 17.0 | 1.06779 | 1.06968 | 9.45 |
| 8.2 | 1.03076 | 1.03258 | 4.58 | 17.2 | 1.06866 | 1.07055 | 9.56 |
| 8.4 | 1.03157 | 1.03340 | 4.69 | 17.4 | 1.06953 | 1.07142 | 9.67 |
| 8.6 | 1.03239 | 1.03422 | 4.80 | 17.6 | 1.07040 | 1.07229 | 9.78 |
| 8.8 | 1.03321 | 1.03504 | 4.91 | 17.8 | 1.07127 | 1.07317 | 9.89 |

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[1] Bur. Standards Circ. **44,** 1918, p. 151.

2 *Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.*—Continued.

| DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | SPECIFIC GRAVITY AT 20/4°C. | SPECIFIC GRAVITY AT 20/20°C. | DEGREES BAUME (MODULUS 145) | DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | SPECIFIC GRAVITY AT 20/4°C. | SPECIFIC GRAVITY AT 20/20°C. | DEGREES BAUME (MODULUS 145) |
|---|---|---|---|---|---|---|---|
| 18.0 | 1.07215 | 1.07404 | 10.00 | 27.0 | 1.11283 | 1.11480 | 14.93 |
| 18.2 | 1.07302 | 1.07492 | 10.11 | 27.2 | 1.11376 | 1.11573 | 15.04 |
| 18.4 | 1.07390 | 1.07580 | 10.22 | 27.4 | 1.11470 | 1.11667 | 15.15 |
| 18.6 | 1.07478 | 1.07668 | 10.33 | 27.6 | 1.11564 | 1.11761 | 15.26 |
| 18.8 | 1.07566 | 1.07756 | 10.44 | 27.8 | 1.11657 | 1.11855 | 15.37 |
| 19.0 | 1.07654 | 1.07844 | 10.55 | 28.0 | 1.11751 | 1.11949 | 15.48 |
| 19.2 | 1.07742 | 1.07932 | 10.66 | 28.2 | 1.11845 | 1.12043 | 15.59 |
| 19.4 | 1.07830 | 1.08021 | 10.77 | 28.4 | 1.11940 | 1.12138 | 15.69 |
| 19.6 | 1.07919 | 1.08110 | 10.88 | 28.6 | 1.12034 | 1.12232 | 15.80 |
| 19.8 | 1.08007 | 1.08198 | 10.99 | 28.8 | 1.12128 | 1.12327 | 15.91 |
| 20.0 | 1.08096 | 1.08287 | 11.10 | 29.0 | 1.12223 | 1.12422 | 16.02 |
| 20.2 | 1.08185 | 1.08376 | 11.21 | 29.2 | 1.12318 | 1.12517 | 16.13 |
| 20.4 | 1.08274 | 1.08465 | 11.32 | 29.4 | 1.12413 | 1.12612 | 16.24 |
| 20.6 | 1.08363 | 1.08554 | 11.43 | 29.6 | 1.12508 | 1.12707 | 16.35 |
| 20.8 | 1.08452 | 1.08644 | 11.54 | 29.8 | 1.12603 | 1.12802 | 16.46 |
| 21.0 | 1.07541 | 1.08733 | 11.65 | 30.0 | 1.12698 | 1.12898 | 16.57 |
| 21.2 | 1.08631 | 1.08823 | 11.76 | 30.2 | 1.12794 | 1.12993 | 16.67 |
| 21.4 | 1.08720 | 1.08913 | 11.87 | 30.4 | 1.12890 | 1.13089 | 16.78 |
| 21.6 | 1.08810 | 1.09003 | 11.98 | 30.6 | 1.12985 | 1.13185 | 16.89 |
| 21.8 | 1.08900 | 1.09093 | 12.09 | 30.8 | 1.13081 | 1.13281 | 17.00 |
| 22.0 | 1.08990 | 1.09183 | 12.20 | 31.0 | 1.13177 | 1.13378 | 17.11 |
| 22.2 | 1.09080 | 1.09273 | 12.31 | 31.2 | 1.13274 | 1.13474 | 17.22 |
| 22.4 | 1.09170 | 1.09364 | 12.42 | 31.4 | 1.13370 | 1.13570 | 17.33 |
| 22.6 | 1.09261 | 1.09454 | 12.52 | 31.6 | 1.13466 | 1.13667 | 17.43 |
| 22.8 | 1.09351 | 1.09545 | 12.63 | 31.8 | 1.13563 | 1.13764 | 17.54 |
| 23.0 | 1.09442 | 1.09636 | 12.74 | 32.0 | 1.13660 | 1.13861 | 17.65 |
| 23.2 | 1.09533 | 1.09727 | 12.85 | 32.2 | 1.13756 | 1.13958 | 17.76 |
| 23.4 | 1.09624 | 1.09818 | 12.96 | 32.4 | 1.13853 | 1.14055 | 17.87 |
| 23.6 | 1.09715 | 1.09909 | 13.07 | 32.6 | 1.13951 | 1.14152 | 17.98 |
| 23.8 | 1.09806 | 1.10000 | 13.18 | 32.8 | 1.14048 | 1.14250 | 18.08 |
| 24.0 | 1.09897 | 1.10092 | 13.29 | 33.0 | 1.14145 | 1.14347 | 18.19 |
| 24.2 | 1.09989 | 1.10183 | 13.40 | 33.2 | 1.14243 | 1.14445 | 18.30 |
| 24.4 | 1.10080 | 1.10275 | 13.51 | 33.4 | 1.14340 | 1.14543 | 18.41 |
| 24.6 | 1.10172 | 1.10367 | 13.62 | 33.6 | 1.14438 | 1.14641 | 18.52 |
| 24.8 | 1.10264 | 1.10459 | 13.73 | 33.8 | 1.14536 | 1.14739 | 18.63 |
| 25.0 | 1.10356 | 1.10551 | 13.84 | 34.0 | 1.14634 | 1.14837 | 18.73 |
| 25.2 | 1.10448 | 1.10643 | 13.95 | 34.2 | 1.14733 | 1.14936 | 18.84 |
| 25.4 | 1.10540 | 1.10736 | 14.06 | 34.4 | 1.14831 | 1.15034 | 18.95 |
| 25.6 | 1.10632 | 1.10828 | 14.17 | 34.6 | 1.14930 | 1.15133 | 19.06 |
| 25.8 | 1.10725 | 1.10921 | 14.28 | 34.8 | 1.15029 | 1.15232 | 19.17 |
| 26.0 | 1.10818 | 1.11014 | 14.39 | 35.0 | 1.15128 | 1.15331 | 19.28 |
| 26.2 | 1.10910 | 1.11106 | 14.49 | 35.2 | 1.15226 | 1.15430 | 19.38 |
| 26.4 | 1.11003 | 1.11200 | 14.60 | 35.4 | 1.15326 | 1.15530 | 19.49 |
| 26.6 | 1.11096 | 1.11293 | 14.71 | 35.6 | 1.15425 | 1.15629 | 19.60 |
| 26.8 | 1.11190 | 1.11386 | 14.82 | 35.8 | 1.15524 | 1.15729 | 19.71 |

*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.*—Continued. 2

| DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | SPECIFIC GRAVITY AT 20/4°C. | SPECIFIC GRAVITY AT 20/20°C. | DEGREES BAUME (MODULUS 145) | DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | SPECIFIC GRAVITY AT 20/4°C. | SPECIFIC GRAVITY AT 20/20°C. | DEGREES BAUME (MODULUS 145) |
|---|---|---|---|---|---|---|---|
| 36.0 | 1.15624 | 1.15828 | 19.81 | 45.0 | 1.20254 | 1.20467 | 24.63 |
| 36.2 | 1.15724 | 1.15928 | 19.92 | 45.2 | 1.20360 | 1.20573 | 24.74 |
| 36.4 | 1.15823 | 1.16028 | 20.03 | 45.4 | 1.20467 | 1.20680 | 24.85 |
| 36.6 | 1.15923 | 1.16128 | 20.14 | 45.6 | 1.20573 | 1.20787 | 24.95 |
| 36.8 | 1.16023 | 1.16228 | 20.25 | 45.8 | 1.20680 | 1.20894 | 25.06 |
| 37.0 | 1.16124 | 1.16329 | 20.35 | 46.0 | 1.20787 | 1.21001 | 25.17 |
| 37.2 | 1.16224 | 1.16430 | 20.46 | 46.2 | 1.20894 | 1.21108 | 25.27 |
| 37.4 | 1.16324 | 1.16530 | 20.57 | 46.4 | 1.21001 | 1.21215 | 25.38 |
| 37.6 | 1.16425 | 1.16631 | 20.68 | 46.6 | 1.21109 | 1.21323 | 25.48 |
| 37.8 | 1.16526 | 1.16732 | 20.78 | 46.8 | 1.21216 | 1.21431 | 25.59 |
| 38.0 | 1.16627 | 1.16833 | 20.89 | 47.0 | 1.21324 | 1.21538 | 25.70 |
| 38.2 | 1.16728 | 1.16934 | 21.00 | 47.2 | 1.21432 | 1.21646 | 25.80 |
| 38.4 | 1.16829 | 1.17036 | 21.11 | 47.4 | 1.21540 | 1.21755 | 25.91 |
| 38.6 | 1.16931 | 1.17138 | 21.21 | 47.6 | 1.21648 | 1.21863 | 26.01 |
| 38.8 | 1.17032 | 1.17239 | 21.32 | 47.8 | 1.21756 | 1.21971 | 26.12 |
| 39.0 | 1.17134 | 1.17341 | 21.43 | 48.0 | 1.21864 | 1.22080 | 26.23 |
| 39.2 | 1.17236 | 1.17443 | 21.54 | 48.2 | 1.21973 | 1.22189 | 26.33 |
| 39.4 | 1.17338 | 1.17545 | 21.64 | 48.4 | 1.22082 | 1.22298 | 26.44 |
| 39.6 | 1.17440 | 1.17648 | 21.75 | 48.6 | 1.22190 | 1.22406 | 26.54 |
| 39.8 | 1.17542 | 1.17750 | 21.86 | 48.8 | 1.22300 | 1.22516 | 26.65 |
| 40.0 | 1.17645 | 1.17853 | 21.97 | 49.0 | 1.22409 | 1.22625 | 26.75 |
| 40.2 | 1.17747 | 1.17956 | 22.07 | 49.2 | 1.22518 | 1.22735 | 26.86 |
| 40.4 | 1.17850 | 1.18058 | 22.18 | 49.4 | 1.22627 | 1.22844 | 26.96 |
| 40.6 | 1.17953 | 1.18162 | 22.29 | 49.6 | 1.22737 | 1.22954 | 27.07 |
| 40.8 | 1.18056 | 1.18265 | 22.39 | 49.8 | 1.22847 | 1.23064 | 27.18 |
| 41.0 | 1.18159 | 1.18368 | 22.50 | 50.0 | 1.22957 | 1.23174 | 27.28 |
| 41.2 | 1.18262 | 1.18472 | 22.61 | 50.2 | 1.23067 | 1.23284 | 27.39 |
| 41.4 | 1.18366 | 1.18575 | 22.72 | 50.4 | 1.23177 | 1.23395 | 27.49 |
| 41.6 | 1.18470 | 1.18679 | 22.82 | 50.6 | 1.23287 | 1.23506 | 27.60 |
| 41.8 | 1.18573 | 1.18783 | 22.93 | 50.8 | 1.23398 | 1.23616 | 27.70 |
| 42.0 | 1.18677 | 1.18887 | 23.04 | 51.0 | 1.23508 | 1.23727 | 27.81 |
| 42.2 | 1.18781 | 1.18992 | 23.14 | 51.2 | 1.23619 | 1.23838 | 27.91 |
| 42.4 | 1.18886 | 1.19096 | 23.25 | 51.4 | 1.23730 | 1.23949 | 28.02 |
| 42.6 | 1.18990 | 1.19201 | 23.36 | 51.6 | 1.23841 | 1.24060 | 28.12 |
| 42.8 | 1.19095 | 1.19305 | 23.46 | 51.8 | 1.23953 | 1.24172 | 28.23 |
| 43.0 | 1.19199 | 1.19410 | 23.57 | 52.0 | 1.24064 | 1.24284 | 28.33 |
| 43.2 | 1.19304 | 1.19515 | 23.68 | 52.2 | 1.24176 | 1.24395 | 28.44 |
| 43.4 | 1.19409 | 1.19620 | 23.78 | 52.4 | 1.24287 | 1.24507 | 28.54 |
| 43.6 | 1.19514 | 1.19726 | 23.89 | 52.6 | 1.24399 | 1.24619 | 28.65 |
| 43.8 | 1.19619 | 1.19831 | 24.00 | 52.8 | 1.24511 | 1.24731 | 28.75 |
| 44.0 | 1.19725 | 1.19936 | 24.10 | 53.0 | 1.24623 | 1.24844 | 28.86 |
| 44.2 | 1.19830 | 1.20042 | 24.21 | 53.2 | 1.24736 | 1.24956 | 28.96 |
| 44.4 | 1.19936 | 1.20148 | 24.32 | 53.4 | 1.24848 | 1.25069 | 29.06 |
| 44.6 | 1.20042 | 1.20254 | 24.42 | 53.6 | 1.24961 | 1.25182 | 29.17 |
| 44.8 | 1.20148 | 1.20360 | 24.53 | 53.8 | 1.25074 | 1.25295 | 29.27 |

**2** *Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.*—Continued.

| DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | SPECIFIC GRAVITY AT 20/4°C. | SPECIFIC GRAVITY AT 20/20°C. | DEGREES BAUMÉ (MODULUS 145) | DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | SPECIFIC GRAVITY AT 20/4°C. | SPECIFIC GRAVITY AT 20/20°C. | DEGREES BAUMÉ (MODULUS 145) |
|---|---|---|---|---|---|---|---|
| 54.0 | 1.25187 | 1.25408 | 29.38 | 63.0 | 1.30427 | 1.30657 | 34.02 |
| 54.2 | 1.25300 | 1.25521 | 29.48 | 63.2 | 1.30547 | 1.30778 | 34.12 |
| 54.4 | 1.25413 | 1.25635 | 29.59 | 63.4 | 1.30667 | 1.30898 | 34.23 |
| 54.6 | 1.25526 | 1.25748 | 29.69 | 63.6 | 1.30787 | 1.31019 | 34.33 |
| 54.8 | 1.25640 | 1.25862 | 29.80 | 63.8 | 1.30908 | 1.31139 | 34.43 |
| 55.0 | 1.25754 | 1.25976 | 29.90 | 64.0 | 1.31028 | 1.31260 | 34.53 |
| 55.2 | 1.25867 | 1.26090 | 30.00 | 64.2 | 1.31149 | 1.31381 | 34.63 |
| 55.4 | 1.25982 | 1.26204 | 30.11 | 64.4 | 1.31270 | 1.31502 | 34.74 |
| 55.6 | 1.26096 | 1.26319 | 30.21 | 64.6 | 1.31391 | 1.31623 | 34.84 |
| 55.8 | 1.26210 | 1.26433 | 30.32 | 64.8 | 1.31512 | 1.31745 | 34.94 |
| 56.0 | 1.26324 | 1.26548 | 30.42 | 65.0 | 1.31633 | 1.31866 | 35.04 |
| 56.2 | 1.26439 | 1.26663 | 30.52 | 65.2 | 1.31755 | 1.31988 | 35.14 |
| 56.4 | 1.26554 | 1.26778 | 30.63 | 65.4 | 1.31877 | 1.32110 | 35.24 |
| 56.6 | 1.26669 | 1.26893 | 30.73 | 65.6 | 1.31998 | 1.32232 | 35.34 |
| 56.8 | 1.26784 | 1.27008 | 30.83 | 65.8 | 1.32120 | 1.32354 | 35.45 |
| 57.0 | 1.26899 | 1.27123 | 30.94 | 66.0 | 1.32242 | 1.32476 | 35.55 |
| 57.2 | 1.27014 | 1.27239 | 31.04 | 66.2 | 1.32365 | 1.32599 | 35.65 |
| 57.4 | 1.27130 | 1.27355 | 31.15 | 66.4 | 1.32487 | 1.32722 | 35.75 |
| 57.6 | 1.27246 | 1.27471 | 31.25 | 66.6 | 1.32610 | 1.32844 | 35.85 |
| 57.8 | 1.27361 | 1.27587 | 31.35 | 66.8 | 1.32732 | 1.32967 | 35.95 |
| 58.0 | 1.27477 | 1.27703 | 31.46 | 67.0 | 1.32855 | 1.33090 | 36.05 |
| 58.2 | 1.27594 | 1.27819 | 31.56 | 67.2 | 1.32978 | 1.33214 | 36.15 |
| 58.4 | 1.27710 | 1.27936 | 31.66 | 67.4 | 1.33102 | 1.33337 | 36.25 |
| 58.6 | 1.27826 | 1.28052 | 31.76 | 67.6 | 1.33225 | 1.33460 | 36.35 |
| 58.8 | 1.27943 | 1.28169 | 31.87 | 67.8 | 1.33348 | 1.33584 | 36.45 |
| 59.0 | 1.28060 | 1.28286 | 31.97 | 68.0 | 1.33472 | 1.33708 | 36.55 |
| 59.2 | 1.28176 | 1.28404 | 32.07 | 68.2 | 1.33596 | 1.33832 | 36.66 |
| 59.4 | 1.28294 | 1.28520 | 32.18 | 68.4 | 1.33720 | 1.33957 | 36.76 |
| 59.6 | 1.28411 | 1.28638 | 32.28 | 68.6 | 1.33844 | 1.34081 | 36.86 |
| 59.8 | 1.28528 | 1.28755 | 32.38 | 68.8 | 1.33968 | 1.34205 | 36.96 |
| 60.0 | 1.28646 | 1.28873 | 32.49 | 69.0 | 1.34093 | 1.34330 | 37.06 |
| 60.2 | 1.28763 | 1.28991 | 32.59 | 69.2 | 1.34217 | 1.34455 | 37.16 |
| 60.4 | 1.28881 | 1.29109 | 32.69 | 69.4 | 1.34342 | 1.34580 | 37.26 |
| 60.6 | 1.28999 | 1.29227 | 32.79 | 69.6 | 1.34467 | 1.34705 | 37.36 |
| 60.8 | 1.29117 | 1.29346 | 32.90 | 69.8 | 1.34592 | 1.34830 | 37.46 |
| 61.0 | 1.29235 | 1.29464 | 33.00 | 70.0 | 1.34717 | 1.34956 | 37.56 |
| 61.2 | 1.29354 | 1.29583 | 33.10 | 70.2 | 1.34843 | 1.35081 | 37.66 |
| 61.4 | 1.29472 | 1.29701 | 33.20 | 70.4 | 1.34968 | 1.35207 | 37.76 |
| 61.6 | 1.29591 | 1.29820 | 33.31 | 70.6 | 1.35094 | 1.35333 | 37.86 |
| 61.8 | 1.29710 | 1.29940 | 33.41 | 70.8 | 1.35220 | 1.35459 | 37.96 |
| 62.0 | 1.29829 | 1.30059 | 33.51 | 71.0 | 1.35346 | 1.35585 | 38.06 |
| 62.2 | 1.29948 | 1.30178 | 33.61 | 71.2 | 1.35472 | 1.35711 | 38.16 |
| 62.4 | 1.30068 | 1.30298 | 33.72 | 71.4 | 1.35598 | 1.35838 | 38.26 |
| 62.6 | 1.30187 | 1.30418 | 33.82 | 71.6 | 1.35724 | 1.35964 | 38.35 |
| 62.8 | 1.30307 | 1.30537 | 33.92 | 71.8 | 1.35851 | 1.36091 | 38.45 |

*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.*—Continued. 2

| DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | SPECIFIC GRAVITY AT 20/4°C. | SPECIFIC GRAVITY AT 20/20°C. | DEGREES BAUME (MODULUS 145) | DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | SPECIFIC GRAVITY AT 20/4°C. | SPECIFIC GRAVITY AT 20/20°C. | DEGREES BAUME (MODULUS 145) |
|---|---|---|---|---|---|---|---|
| 72.0 | 1.35978 | 1.36218 | 38.55 | 81.0 | 1.41837 | 1.42088 | 42.95 |
| 72.2 | 1.36105 | 1.36346 | 38.65 | 81.2 | 1.41971 | 1.42222 | 43.05 |
| 72.4 | 1.36232 | 1.36473 | 38.75 | 81.4 | 1.42105 | 1.42356 | 43.14 |
| 72.6 | 1.36359 | 1.36600 | 38.85 | 81.6 | 1.42239 | 1.42490 | 43.24 |
| 72.8 | 1.36486 | 1.36728 | 38.95 | 81.8 | 1.42373 | 1.42625 | 43.33 |
| 73.0 | 1.36614 | 1.36856 | 39.05 | 82.0 | 1.42507 | 1.42759 | 43.43 |
| 73.2 | 1.36742 | 1.36983 | 39.15 | 82.2 | 1.42642 | 1.42894 | 43.53 |
| 73.4 | 1.36869 | 1.37111 | 39.25 | 82.4 | 1.42776 | 1.43029 | 43.62 |
| 73.6 | 1.36997 | 1.37240 | 39.35 | 82.6 | 1.42911 | 1.43164 | 43.72 |
| 73.8 | 1.37125 | 1.37368 | 39.44 | 82.8 | 1.43046 | 1.43298 | 43.81 |
| 74.0 | 1.37254 | 1.37496 | 39.54 | 83.0 | 1.43181 | 1.43434 | 43.91 |
| 74.2 | 1.37382 | 1.37625 | 39.64 | 83.2 | 1.43316 | 1.43569 | 44.00 |
| 74.4 | 1.37510 | 1.37754 | 39.74 | 83.4 | 1.43451 | 1.43705 | 44.10 |
| 74.6 | 1.37639 | 1.37883 | 39.84 | 83.6 | 1.43587 | 1.43841 | 44.19 |
| 74.8 | 1.37768 | 1.38012 | 39.94 | 83.8 | 1.43722 | 1.43976 | 44.29 |
| 75.0 | 1.37897 | 1.38141 | 40.03 | 84.0 | 1.43858 | 1.44112 | 44.38 |
| 75.2 | 1.38026 | 1.38270 | 40.13 | 84.2 | 1.43994 | 1.44249 | 44.48 |
| 75.4 | 1.38156 | 1.38400 | 40.23 | 84.4 | 1.44130 | 1.44385 | 44.57 |
| 75.6 | 1.38285 | 1.38530 | 40.33 | 84.6 | 1.44266 | 1.44521 | 44.67 |
| 75.8 | 1.38415 | 1.38660 | 40.43 | 84.8 | 1.44402 | 1.44658 | 44.76 |
| 76.0 | 1.38545 | 1.38790 | 40.53 | 85.0 | 1.44539 | 1.44794 | 44.86 |
| 76.2 | 1.38675 | 1.38920 | 40.62 | 85.2 | 1.44675 | 1.44931 | 44.95 |
| 76.4 | 1.38805 | 1.39050 | 40.72 | 85.4 | 1.44812 | 1.45068 | 45.05 |
| 76.6 | 1.38935 | 1.39180 | 40.82 | 85.6 | 1.44949 | 1.45205 | 45.14 |
| 76.8 | 1.39065 | 1.39311 | 40.92 | 85.8 | 1.45086 | 1.45343 | 45.24 |
| 77.0 | 1.39196 | 1.39442 | 41.01 | 86.0 | 1.45223 | 1.45480 | 45.33 |
| 77.2 | 1.39326 | 1.39573 | 41.11 | 86.2 | 1.45360 | 1.45618 | 45.42 |
| 77.4 | 1.39457 | 1.39704 | 41.21 | 86.4 | 1.45498 | 1.45755 | 45.52 |
| 77.6 | 1.39588 | 1.39835 | 41.31 | 86.6 | 1.45636 | 1.45893 | 45.61 |
| 77.8 | 1.39719 | 1.39966 | 41.40 | 86.8 | 1.45773 | 1.46031 | 45.71 |
| 78.0 | 1.39850 | 1.40098 | 41.50 | 87.0 | 1.45911 | 1.46170 | 45.80 |
| 78.2 | 1.39982 | 1.40230 | 41.60 | 87.2 | 1.46050 | 1.46308 | 45.89 |
| 78.4 | 1.40113 | 1.40361 | 41.70 | 87.4 | 1.46188 | 1.46446 | 45.99 |
| 78.6 | 1.40245 | 1.40493 | 41.79 | 87.6 | 1.46326 | 1.46585 | 46.08 |
| 78.8 | 1.40377 | 1.40625 | 41.89 | 87.8 | 1.46464 | 1.46724 | 46.17 |
| 79.0 | 1.40509 | 1.40758 | 41.99 | 88.0 | 1.46603 | 1.46862 | 46.27 |
| 79.2 | 1.40641 | 1.40890 | 42.08 | 88.2 | 1.46742 | 1.47002 | 46.36 |
| 79.4 | 1.40774 | 1.41023 | 42.18 | 88.4 | 1.46881 | 1.47141 | 46.45 |
| 79.6 | 1.40906 | 1.41155 | 42.28 | 88.6 | 1.47020 | 1.47280 | 46.55 |
| 79.8 | 1.41039 | 1.41288 | 42.37 | 88.8 | 1.47159 | 1.47420 | 46.64 |
| 80.0 | 1.41172 | 1.41421 | 42.47 | 89.0 | 1.47299 | 1.47559 | 46.73 |
| 80.2 | 1.41304 | 1.41554 | 42.57 | 89.2 | 1.47438 | 1.47699 | 46.83 |
| 80.4 | 1.41437 | 1.41688 | 42.66 | 89.4 | 1.47578 | 1.47839 | 46.92 |
| 80.6 | 1.41571 | 1.41821 | 42.76 | 89.6 | 1.47718 | 1.47979 | 47.01 |
| 80.8 | 1.41704 | 1.41955 | 42.85 | 89.8 | 1.47858 | 1.48119 | 47.11 |

2 *Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.*—Continued.

| DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | SPECIFIC GRAVITY AT 20/4°C. | SPECIFIC GRAVITY AT 20/20°C. | DEGREES BAUME (MODULUS 145) | DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | SPECIFIC GRAVITY AT 20/4°C. | SPECIFIC GRAVITY AT 20/20°C. | DEGREES BAUME (MODULUS 145) |
|---|---|---|---|---|---|---|---|
| 90.0 | 1.47998 | 1.48259 | 47.20 | 95.0 | 1.51546 | 1.51814 | 49.49 |
| 90.2 | 1.48138 | 1.48400 | 47.29 | 95.2 | 1.51689 | 1.51958 | 49.58 |
| 90.4 | 1.48278 | 1.48540 | 47.38 | 95.4 | 1.51833 | 1.52102 | 49.67 |
| 90.6 | 1.48419 | 1.48681 | 47.48 | 95.6 | 1.51977 | 1.52246 | 49.76 |
| 90.8 | 1.48559 | 1.48822 | 47.57 | 95.8 | 1.52121 | 1.52390 | 49.85 |
| 91.0 | 1.48700 | 1.48963 | 47.66 | 96.0 | 1.52266 | 1.52535 | 49.94 |
| 91.2 | 1.48841 | 1.49104 | 47.75 | 96.2 | 1.52410 | 1.52680 | 50.03 |
| 91.4 | 1.48982 | 1.49246 | 47.84 | 96.4 | 1.52555 | 1.52824 | 50.12 |
| 91.6 | 1.49123 | 1.49387 | 47.94 | 96.6 | 1.52699 | 1.52969 | 50.21 |
| 91.8 | 1.49265 | 1.49529 | 48.03 | 96.8 | 1.52844 | 1.53114 | 50.30 |
| 92.0 | 1.49406 | 1.49671 | 48.12 | 97.0 | 1.52989 | 1.53260 | 50.39 |
| 92.2 | 1.49548 | 1.49812 | 48.21 | 97.2 | 1.53134 | 1.53405 | 50.48 |
| 92.4 | 1.49690 | 1.49954 | 48.30 | 97.4 | 1.53279 | 1.53551 | 50.57 |
| 92.6 | 1.49832 | 1.50097 | 48.40 | 97.6 | 1.53425 | 1.53696 | 50.66 |
| 92.8 | 1.49974 | 1.50239 | 48.49 | 97.8 | 1.53570 | 1.53842 | 50.75 |
| 93.0 | 1.50116 | 1.50381 | 48.58 | 98.0 | 1.53716 | 1.53988 | 50.84 |
| 93.2 | 1.50258 | 1.50524 | 48.67 | 98.2 | 1.53862 | 1.54134 | 50.93 |
| 93.4 | 1.50401 | 1.50667 | 48.76 | 98.4 | 1.54008 | 1.54280 | 51.02 |
| 93.6 | 1.50543 | 1.50810 | 48.85 | 98.6 | 1.54154 | 1.54426 | 51.10 |
| 93.8 | 1.50686 | 1.50952 | 48.94 | 98.8 | 1.54300 | 1.54573 | 51.19 |
| 94.0 | 1.50829 | 1.51096 | 49.03 | 99.0 | 1.54446 | 1.54719 | 51.28 |
| 94.2 | 1.50972 | 1.51239 | 49.12 | 99.2 | 1.54593 | 1.54866 | 51.37 |
| 94.4 | 1.51115 | 1.51382 | 49.22 | 99.4 | 1.54739 | 1.55013 | 51.46 |
| 94.6 | 1.51258 | 1.51526 | 49.31 | 99.6 | 1.54886 | 1.55160 | 51.55 |
| 94.8 | 1.51402 | 1.51670 | 49.40 | 99.8 | 1.55033 | 1.55307 | 51.64 |
|  |  |  |  | 100.0 | 1.55180 | 1.55454 | 51.73 |

## *Temperature corrections to readings of saccharometers (standard at 20°C.).* 3

(This table is calculated from the data on thermal expansion of sugar solutions by Plato,[1] and it is assumed that the instrument is of Jena $16^{III}$ glass. The table should be used with caution and only for approximate results when the temperature differs much from the standard temperature or from the temperature of the surrounding air.)

| TEMPERATURE IN DEGREES CENTIGRADE | OBSERVED PERCENTAGE OF SUGAR | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 70 |
| | Subtract— | | | | | | | | | | | | | |
| 0 | 0.30 | 0.49 | 0.65 | 0.77 | 0.89 | 0.99 | 1.08 | 1.16 | 1.24 | 1.31 | 1.37 | 1.41 | 1.44 | 1.49 |
| 5 | 0.36 | 0.47 | 0.56 | 0.65 | 0.73 | 0.80 | 0.86 | 0.91 | 0.97 | 1.01 | 1.05 | 1.08 | 1.10 | 1.14 |
| 10 | 0.32 | 0.38 | 0.43 | 0.48 | 0.52 | 0.57 | 0.60 | 0.64 | 0.67 | 0.70 | 0.72 | 0.74 | 0.75 | 0.77 |
| 11 | 0.31 | 0.35 | 0.40 | 0.44 | 0.48 | 0.51 | 0.55 | 0.58 | 0.60 | 0.63 | 0.65 | 0.66 | 0.68 | 0.70 |
| 12 | 0.29 | 0.32 | 0.36 | 0.40 | 0.43 | 0.46 | 0.50 | 0.52 | 0.54 | 0.56 | 0.58 | 0.59 | 0.60 | 0.62 |
| 13 | 0.26 | 0.29 | 0.32 | 0.35 | 0.38 | 0.41 | 0.44 | 0.46 | 0.48 | 0.49 | 0.51 | 0.52 | 0.53 | 0.55 |
| 14 | 0.24 | 0.26 | 0.29 | 0.31 | 0.34 | 0.36 | 0.38 | 0.40 | 0.41 | 0.42 | 0.44 | 0.45 | 0.46 | 0.47 |
| 15 | 0.20 | 0.22 | 0.24 | 0.26 | 0.28 | 0.30 | 0.32 | 0.33 | 0.34 | 0.36 | 0.36 | 0.37 | 0.38 | 0.39 |
| 16 | 0.17 | 0.18 | 0.20 | 0.22 | 0.23 | 0.25 | 0.26 | 0.27 | 0.28 | 0.28 | 0.29 | 0.30 | 0.31 | 0.32 |
| 17 | 0.13 | 0.14 | 0.15 | 0.16 | 0.18 | 0.19 | 0.20 | 0.20 | 0.21 | 0.21 | 0.22 | 0.23 | 0.23 | 0.24 |
| 18 | 0.09 | 0.10 | 0.10 | 0.11 | 0.12 | 0.13 | 0.13 | 0.14 | 0.14 | 0.14 | 0.15 | 0.15 | 0.15 | 0.16 |
| 19 | 0.05 | 0.05 | 0.05 | 0.06 | 0.06 | 0.06 | 0.07 | 0.07 | 0.07 | 0.07 | 0.08 | 0.08 | 0.08 | 0.08 |
| 17.5 | 0.11 | 0.12 | 0.12 | 0.14 | 0.15 | 0.16 | 0.16 | 0.17 | 0.17 | 0.18 | 0.18 | 0.19 | 0.19 | 0.20 |
| 15.56 (60°F.) | 0.18 | 0.20 | 0.22 | 0.24 | 0.26 | 0.28 | 0.29 | 0.30 | 0.30 | 0.32 | 0.33 | 0.33 | 0.34 | 0.34 |
| | Add— | | | | | | | | | | | | | |
| 21 | 0.04 | 0.05 | 0.06 | 0.06 | 0.06 | 0.07 | 0.07 | 0.07 | 0.07 | 0.08 | 0.08 | 0.08 | 0.08 | 0.09 |
| 22 | 0.10 | 0.10 | 0.11 | 0.12 | 0.12 | 0.13 | 0.14 | 0.14 | 0.15 | 0.15 | 0.16 | 0.16 | 0.16 | 0.16 |
| 23 | 0.16 | 0.16 | 0.17 | 0.17 | 0.19 | 0.20 | 0.21 | 0.21 | 0.22 | 0.23 | 0.24 | 0.24 | 0.24 | 0.24 |
| 24 | 0.21 | 0.22 | 0.23 | 0.24 | 0.26 | 0.27 | 0.28 | 0.29 | 0.30 | 0.31 | 0.32 | 0.32 | 0.32 | 0.32 |
| 25 | 0.27 | 0.28 | 0.30 | 0.31 | 0.32 | 0.34 | 0.35 | 0.36 | 0.38 | 0.38 | 0.39 | 0.39 | 0.40 | 0.39 |
| 26 | 0.33 | 0.34 | 0.36 | 0.37 | 0.40 | 0.40 | 0.42 | 0.44 | 0.46 | 0.47 | 0.47 | 0.48 | 0.48 | 0.48 |
| 27 | 0.40 | 0.41 | 0.42 | 0.44 | 0.46 | 0.48 | 0.50 | 0.52 | 0.54 | 0.54 | 0.55 | 0.56 | 0.56 | 0.56 |
| 28 | 0.46 | 0.47 | 0.49 | 0.51 | 0.54 | 0.56 | 0.58 | 0.60 | 0.61 | 0.62 | 0.63 | 0.64 | 0.64 | 0.64 |
| 29 | 0.54 | 0.55 | 0.56 | 0.59 | 0.61 | 0.63 | 0.66 | 0.68 | 0.70 | 0.70 | 0.71 | 0.72 | 0.72 | 0.72 |
| 30 | 0.61 | 0.62 | 0.63 | 0.66 | 0.68 | 0.71 | 0.73 | 0.76 | 0.78 | 0.78 | 0.79 | 0.80 | 0.80 | 0.81 |
| 35 | 0.99 | 1.01 | 1.02 | 1.06 | 1.10 | 1.13 | 1.16 | 1.18 | 1.20 | 1.21 | 1.22 | 1.22 | 1.23 | 1.22 |
| 40 | 1.42 | 1.45 | 1.47 | 1.51 | 1.54 | 1.57 | 1.60 | 1.62 | 1.64 | 1.65 | 1.65 | 1.65 | 1.66 | 1.65 |
| 45 | 1.91 | 1.94 | 1.96 | 2.00 | 2.03 | 2.05 | 2.07 | 2.09 | 2.10 | 2.10 | 2.10 | 2.10 | 2.10 | 2.08 |
| 50 | 2.46 | 2.48 | 2.50 | 2.53 | 2.56 | 2.57 | 2.58 | 2.59 | 2.59 | 2.58 | 2.58 | 2.57 | 2.56 | 2.52 |
| 55 | 3.05 | 3.07 | 3.09 | 3.12 | 3.12 | 3.12 | 3.12 | 3.11 | 3.10 | 3.08 | 3.07 | 3.05 | 3.03 | 2.97 |
| 60 | 3.69 | 3.72 | 3.73 | 3.73 | 3.72 | 3.70 | 3.67 | 3.65 | 3.62 | 3.60 | 3.57 | 3.54 | 3.50 | 3.43 |
| 27.5 | 0.43 | 0.44 | 0.46 | 0.48 | 0.50 | 0.52 | 0.54 | 0.56 | 0.58 | 0.58 | 0.59 | 0.60 | 0.60 | 0.60 |

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[1] Wiss., Abh. der Kaiserlichen Normal-Eichungs-Kommission, Vol. 2, 1900, p. 140.

## 4 *Domke's table of apparent specific gravity of sucrose solutions at 20°C.*

Calculated from the tables of the Kaiserliche Normal-Eichungs-Kommission and accepted by the International Commission for Unifying Methods of Sugar Analysis.

| DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | .0 | .1 | .2 | .3 | .4 | .5 | .6 | .7 | .8 | .9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1.0000 | 1.0004 | 1.0008 | 1.0012 | 1.0016 | 1.0019 | 1.0023 | 1.0027 | 1.0031 | 1.0035 |
| 1 | 1.0039 | 1.0043 | 1.0047 | 1.0051 | 1.0055 | 1.0058 | 1.0062 | 1.0066 | 1.0070 | 1.0074 |
| 2 | 1.0078 | 1.0082 | 1.0086 | 1.0090 | 1.0094 | 1.0098 | 1.0102 | 1.0106 | 1.0109 | 1.0113 |
| 3 | 1.0117 | 1.0121 | 1.0125 | 1.0129 | 1.0133 | 1.0137 | 1.0141 | 1.0145 | 1.0149 | 1.0153 |
| 4 | 1.0157 | 1.0161 | 1.0165 | 1.0169 | 1.0173 | 1.0177 | 1.0181 | 1.0185 | 1.0189 | 1.0193 |
| 5 | 1.0197 | 1.0201 | 1.0205 | 1.0209 | 1.0213 | 1.0217 | 1.0221 | 1.0225 | 1.0229 | 1.0233 |
| 6 | 1.0237 | 1.0241 | 1.0245 | 1.0249 | 1.0253 | 1.0257 | 1.0261 | 1.0265 | 1.0269 | 1.0273 |
| 7 | 1.0277 | 1.0281 | 1.0285 | 1.0289 | 1.0294 | 1.0298 | 1.0302 | 1.0306 | 1.0310 | 1.0314 |
| 8 | 1.0318 | 1.0322 | 1.0326 | 1.0330 | 1.0334 | 1.0338 | 1.0343 | 1.0347 | 1.0351 | 1.0355 |
| 9 | 1.0359 | 1.0363 | 1.0367 | 1.0371 | 1.0375 | 1.0380 | 1.0384 | 1.0388 | 1.0392 | 1.0396 |
| 10 | 1.0400 | 1.0404 | 1.0409 | 1.0413 | 1.0417 | 1.0421 | 1.0425 | 1.0429 | 1.0433 | 1.0438 |
| 11 | 1.0442 | 1.0446 | 1.0450 | 1.0454 | 1.0459 | 1.0463 | 1.0467 | 1.0471 | 4.0475 | 1.0480 |
| 12 | 1.0484 | 1.0488 | 1.0492 | 1.0496 | 1.0501 | 1.0505 | 1.0509 | 1.0513 | 1.0517 | 1.0522 |
| 13 | 1.0526 | 1.0530 | 1.0534 | 1.0539 | 1.0543 | 1.0547 | 1.0551 | 1.0556 | 1.0560 | 1.0564 |
| 14 | 1.0568 | 1.0573 | 1.0577 | 1.0581 | 1.0585 | 1.0589 | 1.0594 | 1.0598 | 1.0603 | 1.0607 |
| 15 | 1.0611 | 1.0615 | 1.0620 | 1.0624 | 1.0628 | 1.0633 | 1.0637 | 1.0641 | 1.0646 | 1.0650 |
| 16 | 1.0654 | 1.0659 | 1.0663 | 1.0667 | 1.0672 | 1.0676 | 1.0680 | 1.0685 | 1.0689 | 1.0693 |
| 17 | 1.0698 | 1.0702 | 1.0706 | 1.0711 | 1.0715 | 1.0719 | 1.0724 | 1.0728 | 1.0733 | 1.0737 |
| 18 | 1.0741 | 1.0746 | 1.0750 | 1.0755 | 1.0759 | 1.0763 | 1.0768 | 1.0772 | 1.0777 | 1.0781 |
| 19 | 1.0785 | 1.0790 | 1.0794 | 1.0799 | 1.0803 | 1.0807 | 1.0812 | 1.0816 | 1.0821 | 1.0825 |
| 20 | 1.0830 | 1.0834 | 1.0839 | 1.0843 | 1.0848 | 1.0852 | 1.0856 | 1.0861 | 1.0865 | 1.0870 |
| 21 | 1.0874 | 1.0879 | 1.0883 | 1.0888 | 1.0892 | 1.0897 | 1.0901 | 1.0905 | 1.0910 | 1.0915 |
| 22 | 1.0919 | 1.0924 | 1.0928 | 1.0933 | 1.0937 | 1.0942 | 1.0946 | 1.0951 | 1.0956 | 1.0960 |
| 23 | 1.0965 | 1.0969 | 1.0974 | 1.0978 | 1.0983 | 1.0987 | 1.0992 | 1.0997 | 1.1001 | 1.1006 |
| 24 | 1.1010 | 1.1015 | 1.1020 | 1.1024 | 1.1029 | 1.1033 | 1.1038 | 1.1043 | 1.1047 | 1.1052 |
| 25 | 1.1056 | 1.1061 | 1.1066 | 1.1070 | 1.1075 | 1.1079 | 1.1084 | 1.1089 | 1.1093 | 1.1098 |
| 26 | 1.1103 | 1.1107 | 1.1112 | 1.1117 | 1.1121 | 1.1126 | 1.1131 | 1.1135 | 1.1140 | 1.1145 |
| 27 | 1.1149 | 1.1154 | 1.1159 | 1.1163 | 1.1168 | 1.1173 | 1.1178 | 1.1182 | 1.1187 | 1.1192 |
| 28 | 1.1196 | 1.1201 | 1.1206 | 1.1210 | 1.1215 | 1.1220 | 1.1225 | 1.1229 | 1.1234 | 1.1239 |
| 29 | 1.1244 | 1.1248 | 1.1253 | 1.1258 | 1.1263 | 1.1267 | 1.1272 | 1.1277 | 1.1282 | 1.1287 |
| 30 | 1.1291 | 1.1296 | 1.1301 | 1.1306 | 1.1311 | 1.1315 | 1.1320 | 1.1325 | 1.1330 | 1.1334 |
| 31 | 1.1339 | 1.1344 | 1.1349 | 1.1354 | 1.1359 | 1.1363 | 1.1368 | 1.1373 | 1.1378 | 1.1383 |
| 32 | 1.1388 | 1.1393 | 1.1397 | 1.1402 | 1.1407 | 1.1412 | 1.1417 | 1.1422 | 1.1427 | 1.1432 |
| 33 | 1.1436 | 1.1441 | 1.1446 | 1.1451 | 1.1456 | 1.1461 | 1.1466 | 1.1471 | 1.1476 | 1.1481 |
| 34 | 1.1486 | 1.1490 | 1.1495 | 1.1500 | 1.1505 | 1.1510 | 1.1515 | 1.1520 | 1.1525 | 1.1530 |
| 35 | 1.1535 | 1.1540 | 1.1545 | 1.1550 | 1.1555 | 1.1560 | 1.1565 | 1.1570 | 1.1575 | 1.1580 |
| 36 | 1.1585 | 1.1590 | 1.1595 | 1.1600 | 1.1605 | 1.1610 | 1.1615 | 1.1620 | 1.1625 | 1.1630 |
| 37 | 1.1635 | 1.1640 | 1.1645 | 1.1650 | 1.1655 | 1.1660 | 1.1665 | 1.1670 | 1.1675 | 1.1680 |
| 38 | 1.1685 | 1.1690 | 1.1696 | 1.1701 | 1.1706 | 1.1711 | 1.1716 | 1.1721 | 1.1726 | 1.1731 |
| 39 | 1.1736 | 1.1741 | 1.1746 | 1.1752 | 1.1757 | 1.1762 | 1.1767 | 1.1772 | 1.1777 | 1.1782 |
| 40 | 1.1787 | 1.1793 | 1.1798 | 1.1803 | 1.1808 | 1.1813 | 1.1818 | 1.1824 | 1.1829 | 1.1834 |
| 41 | 1.1839 | 1.1844 | 1.1849 | 1.1855 | 1.1860 | 1.1865 | 1.1870 | 1.1875 | 1.1881 | 1.1886 |
| 42 | 1.1891 | 1.1896 | 1.1901 | 1.1907 | 1.1912 | 1.1917 | 1.1922 | 1.1928 | 1.1933 | 1.1938 |
| 43 | 1.1943 | 1.1949 | 1.1954 | 1.1959 | 1.1964 | 1.1970 | 1.1975 | 1.1980 | 1.1985 | 1.1991 |
| 44 | 1.1996 | 1.2001 | 1.2007 | 1.2012 | 1.2017 | 1.2023 | 1.2028 | 1.2033 | 1.2039 | 1.2044 |

*Domke's table of apparent specific gravity of sucrose solutions at 20°C.*—Continued. 4

| DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE | .0 | .1 | .2 | .3 | .4 | .5 | .6 | .7 | .8 | .9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 45 | 1.2049 | 1.2054 | 1.2060 | 1.2065 | 1.2070 | 1.2076 | 1.2081 | 1.2087 | 1.2092 | 1.2097 |
| 46 | 1.2102 | 1.2108 | 1.2113 | 1.2118 | 1.2124 | 1.2129 | 1.2135 | 1.2140 | 1.2146 | 1.2151 |
| 47 | 1.2156 | 1.2162 | 1.2167 | 1.2173 | 1.2178 | 1.2184 | 1.2189 | 1.2194 | 1.2200 | 1.2205 |
| 48 | 1.2211 | 1.2216 | 1.2222 | 1.2227 | 1.2232 | 1.2238 | 1.2243 | 1.2249 | 1.2254 | 1.2260 |
| 49 | 1.2265 | 1.2271 | 1.2276 | 1.2282 | 1.2287 | 1.2293 | 1.2298 | 1.2304 | 1.2309 | 1.2315 |
| 50 | 1.2320 | 1.2326 | 1.2331 | 1.2337 | 1.2342 | 1.2348 | 1.2353 | 1.2359 | 1.2364 | 1.2370 |
| 51 | 1.2376 | 1.2381 | 1.2387 | 1.2392 | 1.2398 | 1.2403 | 1.2409 | 1.2415 | 1.2420 | 1.2426 |
| 52 | 1.2431 | 1.2437 | 1.2442 | 1.2448 | 1.2454 | 1.2459 | 1.2465 | 1.2471 | 1.2476 | 1.2482 |
| 53 | 1.2487 | 1.2493 | 1.2499 | 1.2504 | 1.2510 | 1.2516 | 1.2521 | 1.2527 | 1.2533 | 1.2538 |
| 54 | 1.2544 | 1.2550 | 1.2555 | 1.2561 | 1.2567 | 1.2572 | 1.2578 | 1.2584 | 1.2589 | 1.2595 |
| 55 | 1.2601 | 1.2606 | 1.2612 | 1.2618 | 1.2624 | 1.2629 | 1.2635 | 1.2641 | 1.2647 | 1.2652 |
| 56 | 1.2658 | 1.2664 | 1.2670 | 1.2675 | 1.2681 | 1.2687 | 1.2693 | 1.2698 | 1.2704 | 1.2710 |
| 57 | 1.2716 | 1.2721 | 1.2727 | 1.2733 | 1.2739 | 1.2745 | 1.2750 | 1.2756 | 1.2762 | 1.2768 |
| 58 | 1.2774 | 1.2779 | 1.2785 | 1.2791 | 1.2797 | 1.2803 | 1.2809 | 1.2815 | 1.2821 | 1.2826 |
| 59 | 1.2832 | 1.2838 | 1.2844 | 1.2850 | 1.2856 | 1.2861 | 1.2867 | 1.2873 | 1.2879 | 1.2885 |
| 60 | 1.2891 | 1.2897 | 1.2903 | 1.2909 | 1.2914 | 1.2920 | 1.2926 | 1.2932 | 1.2938 | 1.2944 |
| 61 | 1.2950 | 1.2956 | 1.2962 | 1.2968 | 1.2974 | 1.2980 | 1.2986 | 1.2992 | 1.2998 | 1.3004 |
| 62 | 1.3010 | 1.3015 | 1.3021 | 1.3027 | 1.3033 | 1.3039 | 1.3045 | 1.3051 | 1.3057 | 1.3063 |
| 63 | 1.3069 | 1.3075 | 1.3081 | 1.3087 | 1.3093 | 1.3100 | 1.3106 | 1.3112 | 1.3118 | 1.3124 |
| 64 | 1.3130 | 1.3136 | 1.3142 | 1.3148 | 1.3154 | 1.3160 | 1.3166 | 1.3172 | 1.3178 | 1.3184 |
| 65 | 1.3190 | 1.3197 | 1.3203 | 1.3209 | 1.3215 | 1.3221 | 1.3227 | 1.3233 | 1.3239 | 1.3245 |
| 66 | 1.3252 | 1.3258 | 1.3264 | 1.3270 | 1.3276 | 1.3282 | 1.3288 | 1.3295 | 1.3301 | 1.3307 |
| 67 | 1.3313 | 1.3319 | 1.3325 | 1.3332 | 1.3338 | 1.3344 | 1.3350 | 1.3356 | 1.3363 | 1.3369 |
| 68 | 1.3375 | 1.3381 | 1.3387 | 1.3394 | 1.3400 | 1.3406 | 1.3412 | 1.3418 | 1.3425 | 1.3431 |
| 69 | 1.3437 | 1.3443 | 1.3450 | 1.3456 | 1.3462 | 1.3468 | 1.3475 | 1.3481 | 1.3487 | 1.3494 |
| 70 | 1.3500 | 1.3506 | 1.3512 | 1.3519 | 1.3525 | 1.3531 | 1.3538 | 1.3544 | 1.3550 | 1.3557 |
| 71 | 1.3563 | 1.3569 | 1.3575 | 1.3582 | 1.3588 | 1.3594 | 1.3601 | 1.3607 | 1.3614 | 1.3620 |
| 72 | 1.3626 | 1.3633 | 1.3639 | 1.3645 | 1.3652 | 1.3658 | 1.3664 | 1.3671 | 1.3677 | 1.3684 |
| 73 | 1.3690 | 1.3696 | 1.3703 | 1.3709 | 1.3716 | 1.3722 | 1.3729 | 1.3735 | 1.3741 | 1.3748 |
| 74 | 1.3754 | 1.3761 | 1.3767 | 1.3774 | 1.3780 | 1.3786 | 1.3793 | 1.3799 | 1.3806 | 1.3812 |
| 75 | 1.3819 | 1.3825 | 1.3832 | 1.3838 | 1.3845 | 1.3851 | 1.3858 | 1.3864 | 1.3871 | 1.3877 |
| 76 | 1.3884 | 1.3890 | 1.3897 | 1.3903 | 1.3910 | 1.3916 | 1.3923 | 1.3929 | 1.3936 | 1.3942 |
| 77 | 1.3949 | 1.3955 | 1.3962 | 1.3969 | 1.3975 | 1.3982 | 1.3988 | 1.3995 | 1.4001 | 1.4008 |
| 78 | 1.4015 | 1.4021 | 1.4028 | 1.4034 | 1.4041 | 1.4048 | 1.4054 | 1.4061 | 1.4067 | 1.4074 |
| 79 | 1.4081 | 1.4087 | 1.4094 | 1.4101 | 1.4107 | 1.4114 | 1.4121 | 1.4127 | 1.4134 | 1.4140 |
| 80 | 1.4147 | 1.4154 | 1.4160 | 1.4167 | 1.4174 | 1.4180 | 1.4187 | 1.4194 | 1.4201 | 1.4207 |
| 81 | 1.4214 | 1.4221 | 1.4227 | 1.4234 | 1.4241 | 1.4247 | 1.4254 | 1.4261 | 1.4268 | 1.4274 |
| 82 | 1.4281 | 1.4288 | 1.4295 | 1.4301 | 1.4308 | 1.4315 | 1.4322 | 1.4328 | 1.4335 | 1.4342 |
| 83 | 1.4349 | 1.4355 | 1.4362 | 1.4369 | 1.4376 | 1.4383 | 1.4389 | 1.4396 | 1.4403 | 1.4410 |
| 84 | 1.4417 | 1.4423 | 1.4430 | 1.4437 | 1.4444 | 1.4451 | 1.4458 | 1.4464 | 1.4471 | 1.4478 |
| 85 | 1.4485 | 1.4492 | 1.4499 | 1.4505 | 1.4512 | 1.4519 | 1.4526 | 1.4533 | 1.4540 | 1.4547 |
| 86 | 1.4554 | 1.4560 | 1.4567 | 1.4574 | 1.4581 | 1.4588 | 1.4595 | 1.4602 | 1.4609 | 1.4616 |
| 87 | 1.4623 | 1.4629 | 1.4636 | 1.4643 | 1.4650 | 1.4657 | 1.4664 | 1.4671 | 1.4678 | 1.4685 |
| 88 | 1.4692 | 1.4699 | 1.4706 | 1.4713 | 1.4720 | 1.4727 | 1.4734 | 1.4741 | 1.4748 | 1.4755 |
| 89 | 1.4762 | 1.4769 | 1.4776 | 1.4783 | 1.4790 | 1.4797 | 1.4804 | 1.4811 | 1.4818 | 1.4825 |
| 90 | 1.4832 | | | | | | | | | |

**5** *Densities of solutions of cane sugar at 20°C.*

| PER CENT SUGAR | TENTHS OF PER CENT | | | | | PER CENT SUGAR |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | |
| 0 | 0.998234 | 0.998622 | 0.999010 | 0.999398 | 0.999786 | 0 |
| 1 | 1.002120 | 1.002509 | 1.002897 | 1.003286 | 1.003675 | 1 |
| 2 | 1.006015 | 1.006405 | 1.006796 | 1.007188 | 1.007580 | 2 |
| 3 | 1.009934 | 1.010327 | 1.010721 | 1.011115 | 1.011510 | 3 |
| 4 | 1.013881 | 1.014277 | 1.014673 | 1.015070 | 1.015467 | 4 |
| 5 | 1.017854 | 1.018253 | 1.018652 | 1.019052 | 1.019451 | 5 |
| 6 | 1.021855 | 1.022257 | 1.022659 | 1.023061 | 1.023463 | 6 |
| 7 | 1.025885 | 1.026289 | 1.026694 | 1.027099 | 1.027504 | 7 |
| 8 | 1.029942 | 1.030349 | 1.030757 | 1.031165 | 1.031573 | 8 |
| 9 | 1.034029 | 1.034439 | 1.034850 | 1.035260 | 1.035671 | 9 |
| 10 | 1.038143 | 1.038556 | 1.038970 | 1.039383 | 1.039797 | 10 |
| 11 | 1.042288 | 1.042704 | 1.043121 | 1.043537 | 1.043954 | 11 |
| 12 | 1.046462 | 1.046881 | 1.047300 | 1.047720 | 1.048140 | 12 |
| 13 | 1.050665 | 1.051087 | 1.051510 | 1.051933 | 1.052356 | 13 |
| 14 | 1.054900 | 1.055325 | 1.055751 | 1.056176 | 1.056602 | 14 |
| 15 | 1.059165 | 1.059593 | 1.060022 | 1.060451 | 1.060880 | 15 |
| 16 | 1.063460 | 1.063892 | 1.064324 | 1.064756 | 1.065188 | 16 |
| 17 | 1.067789 | 1.068223 | 1.068658 | 1.069093 | 1.069529 | 17 |
| 18 | 1.072147 | 1.072585 | 1.073023 | 1.073461 | 1.073900 | 18 |
| 19 | 1.076537 | 1.076978 | 1.077419 | 1.077860 | 1.078302 | 19 |
| 20 | 1.080959 | 1.081403 | 1.081848 | 1.082292 | 1.082737 | 20 |
| 21 | 1.085414 | 1.085861 | 1.086309 | 1.086757 | 1.087205 | 21 |
| 22 | 1.089900 | 1.090351 | 1.090802 | 1.091253 | 1.091704 | 22 |
| 23 | 1.094420 | 1.094874 | 1.095328 | 1.095782 | 1.096236 | 23 |
| 24 | 1.098971 | 1.099428 | 1.099886 | 1.100344 | 1.100802 | 24 |
| 25 | 1.103557 | 1.104017 | 1.104478 | 1.104938 | 1.105400 | 25 |
| 26 | 1.108175 | 1.108639 | 1.109103 | 1.109568 | 1.110033 | 26 |
| 27 | 1.112828 | 1.113295 | 1.113763 | 1.114229 | 1.114697 | 27 |
| 28 | 1.117512 | 1.117982 | 1.118453 | 1.118923 | 1.119395 | 28 |
| 29 | 1.122231 | 1.122705 | 1.123179 | 1.123653 | 1.124128 | 29 |
| 30 | 1.126984 | 1.127461 | 1.127939 | 1.128417 | 1.128896 | 30 |
| 31 | 1.131773 | 1.132254 | 1.132735 | 1.133216 | 1.133698 | 31 |
| 32 | 1.136596 | 1.137080 | 1.137565 | 1.138049 | 1.138534 | 32 |
| 33 | 1.141453 | 1.141941 | 1.142429 | 1.142916 | 1.143405 | 33 |
| 34 | 1.146345 | 1.146836 | 1.147328 | 1.147820 | 1.148313 | 34 |
| 35 | 1.151275 | 1.151770 | 1.152265 | 1.152760 | 1.153256 | 35 |
| 36 | 1.156238 | 1.156736 | 1.157235 | 1.157733 | 1.158233 | 36 |
| 37 | 1.161236 | 1.161738 | 1.162240 | 1.162742 | 1.163245 | 37 |
| 38 | 1.166269 | 1.166775 | 1.167281 | 1.167786 | 1.168293 | 38 |
| 39 | 1.171340 | 1.171849 | 1.172359 | 1.172869 | 1.173379 | 39 |
| 40 | 1.176447 | 1.176960 | 1.177473 | 1.177987 | 1.178501 | 40 |
| 41 | 1.181592 | 1.182108 | 1.182625 | 1.183142 | 1.183660 | 41 |
| 42 | 1.186773 | 1.187293 | 1.187814 | 1.188335 | 1.188856 | 42 |
| 43 | 1.191993 | 1.192517 | 1.193041 | 1.193565 | 1.194090 | 43 |
| 44 | 1.197247 | 1.197775 | 1.198303 | 1.198832 | 1.199360 | 44 |
| 45 | 1.202540 | 1.203071 | 1.203603 | 1.204136 | 1.204668 | 45 |
| 46 | 1.207870 | 1.208405 | 1.208940 | 1.209477 | 1.210013 | 46 |
| 47 | 1.213238 | 1.213777 | 1.214317 | 1.214856 | 1.215395 | 47 |
| 48 | 1.218643 | 1.219185 | 1.219729 | 1.220272 | 1.220815 | 48 |
| 49 | 1.224086 | 1.224632 | 1.225180 | 1.225727 | 1.226274 | 49 |

*Densities of solutions of cane sugar at 20°C.*—Continued. **5**

| PER CENT SUGAR | TENTHS OF PER CENT | | | | | PER CENT SUGAR |
|---|---|---|---|---|---|---|
| | 5 | 6 | 7 | 8 | 9 | |
| 0 | 1.000174 | 1.000563 | 1.000952 | 1.001342 | 1.001731 | 0 |
| 1 | 1.004064 | 1.004453 | 1.004844 | 1.005234 | 1.005624 | 1 |
| 2 | 1.007972 | 1.008363 | 1.008755 | 1.009148 | 1.009541 | 2 |
| 3 | 1.011904 | 1.012298 | 1.012694 | 1.013089 | 1.013485 | 3 |
| 4 | 1.015864 | 1.016261 | 1.016659 | 1.017058 | 1.017456 | 4 |
| 5 | 1.019851 | 1.020251 | 1.020651 | 1.021053 | 1.021454 | 5 |
| 6 | 1.023867 | 1.024270 | 1.024673 | 1.025077 | 1.025481 | 6 |
| 7 | 1.027910 | 1.028316 | 1.028722 | 1.029128 | 1.029535 | 7 |
| 8 | 1.031982 | 1.032391 | 1.032800 | 1.033209 | 1.033619 | 8 |
| 9 | 1.036082 | 1.036494 | 1.036906 | 1.037318 | 1.037730 | 9 |
| 10 | 1.040212 | 1.040626 | 1.041041 | 1.041456 | 1.041872 | 10 |
| 11 | 1.044370 | 1.044788 | 1.045206 | 1.045625 | 1.046043 | 11 |
| 12 | 1.048559 | 1.048980 | 1.049401 | 1.049822 | 1.050243 | 12 |
| 13 | 1.052778 | 1.053202 | 1.053626 | 1.054050 | 1.054475 | 13 |
| 14 | 1.057029 | 1.057455 | 1.057882 | 1.058310 | 1.058737 | 14 |
| 15 | 1.061308 | 1.061738 | 1.062168 | 1.062598 | 1.063029 | 15 |
| 16 | 1.065621 | 1.066054 | 1.066487 | 1.066921 | 1.067355 | 16 |
| 17 | 1.069964 | 1.070400 | 1.070836 | 1.071273 | 1.071710 | 17 |
| 18 | 1.074338 | 1.074777 | 1.075217 | 1.075657 | 1.076097 | 18 |
| 19 | 1.078744 | 1.079187 | 1.079629 | 1.080072 | 1.080515 | 19 |
| 20 | 1.083182 | 1.083628 | 1.084074 | 1.084520 | 1.084967 | 20 |
| 21 | 1.087652 | 1.088101 | 1.088550 | 1.089000 | 1.089450 | 21 |
| 22 | 1.092155 | 1.092607 | 1.093060 | 1.093513 | 1.093966 | 22 |
| 23 | 1.096691 | 1.097147 | 1.097603 | 1.098058 | 1.098514 | 23 |
| 24 | 1.101259 | 1.101718 | 1.102177 | 1.102637 | 1.103097 | 24 |
| 25 | 1.105862 | 1.106324 | 1.106786 | 1.107248 | 1.107711 | 25 |
| 26 | 1.110497 | 1.110963 | 1.111429 | 1.111895 | 1.112361 | 26 |
| 27 | 1.115166 | 1.115635 | 1.116104 | 1.116572 | 1.117042 | 27 |
| 28 | 1.119867 | 1.120339 | 1.120812 | 1.121284 | 1.121757 | 28 |
| 29 | 1.124603 | 1.125079 | 1.125555 | 1.126030 | 1.126507 | 29 |
| 30 | 1.129374 | 1.129853 | 1.130332 | 1.130812 | 1.131292 | 30 |
| 31 | 1.134180 | 1.134663 | 1.135146 | 1.135628 | 1.136112 | 31 |
| 32 | 1.139020 | 1.139506 | 1.139993 | 1.140479 | 1.140966 | 32 |
| 33 | 1.143894 | 1.144384 | 1.144874 | 1.145363 | 1.145854 | 33 |
| 34 | 1.148805 | 1.149298 | 1.149792 | 1.150286 | 1.150780 | 34 |
| 35 | 1.153752 | 1.154249 | 1.154746 | 1.155242 | 1.155740 | 35 |
| 36 | 1.158733 | 1.159233 | 1.159733 | 1.160233 | 1.160734 | 36 |
| 37 | 1.163748 | 1.164252 | 1.164756 | 1.165259 | 1.165764 | 37 |
| 38 | 1.168800 | 1.169307 | 1.169815 | 1.170322 | 1.170831 | 38 |
| 39 | 1.173889 | 1.174400 | 1.174911 | 1.175423 | 1.175935 | 39 |
| 40 | 1.179014 | 1.179527 | 1.180044 | 1.180560 | 1.181076 | 40 |
| 41 | 1.184178 | 1.184696 | 1.185215 | 1.185734 | 1.186253 | 41 |
| 42 | 1.189379 | 1.189901 | 1.190423 | 1.190946 | 1.191469 | 42 |
| 43 | 1.194616 | 1.195141 | 1.195667 | 1.196193 | 1.196720 | 43 |
| 44 | 1.199890 | 1.200420 | 1.200950 | 1.201480 | 1.202010 | 44 |
| 45 | 1.205200 | 1.205733 | 1.206266 | 1.206801 | 1.207335 | 45 |
| 46 | 1.210549 | 1.211086 | 1.211623 | 1.212162 | 1.212700 | 46 |
| 47 | 1.215936 | 1.216476 | 1.217017 | 1.217559 | 1.218101 | 47 |
| 48 | 1.221360 | 1.221904 | 1.222449 | 1.222995 | 1.223540 | 48 |
| 49 | 1.226823 | 1.227371 | 1.227919 | 1.228469 | 1.229018 | 49 |

5

*Densities of solutions of cane sugar at 20°C.*—Continued.

| PER CENT SUGAR | TENTHS OF PER CENT | | | | | PER CENT SUGAR |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | |
| 50 | 1.229567 | 1.230117 | 1.230668 | 1.231219 | 1.231770 | 50 |
| 51 | 1.235085 | 1.235639 | 1.236194 | 1.236748 | 1.237303 | 51 |
| 52 | 1.240641 | 1.241198 | 1.241757 | 1.242315 | 1.242873 | 52 |
| 53 | 1.246234 | 1.246795 | 1.247358 | 1.247920 | 1.248482 | 53 |
| 54 | 1.251866 | 1.252431 | 1.252997 | 1.253563 | 1.254129 | 54 |
| 55 | 1.257535 | 1.258104 | 1.258674 | 1.259244 | 1.259815 | 55 |
| 56 | 1.263243 | 1.263816 | 1.264390 | 1.264963 | 1.265537 | 56 |
| 57 | 1.268989 | 1.269565 | 1.270143 | 1.270720 | 1.271299 | 57 |
| 58 | 1.274774 | 1.275354 | 1.275936 | 1.276517 | 1.277098 | 58 |
| 59 | 1.280595 | 1.281179 | 1.281764 | 1.282349 | 1.282935 | 59 |
| 60 | 1.286456 | 1.287044 | 1.287633 | 1.288222 | 1.288811 | 60 |
| 61 | 1.292354 | 1.292946 | 1.293539 | 1.294131 | 1.294725 | 61 |
| 62 | 1.298291 | 1.298886 | 1.299483 | 1.300079 | 1.300677 | 62 |
| 63 | 1.304267 | 1.304867 | 1.305467 | 1.306068 | 1.306669 | 63 |
| 64 | 1.310282 | 1.310885 | 1.311489 | 1.312093 | 1.312699 | 64 |
| 65 | 1.316334 | 1.316941 | 1.317549 | 1.318157 | 1.318766 | 65 |
| 66 | 1.322425 | 1.323036 | 1.323648 | 1.324259 | 1.324872 | 66 |
| 67 | 1.328554 | 1.329170 | 1.329785 | 1.330401 | 1.331017 | 67 |
| 68 | 1.334722 | 1.335342 | 1.335961 | 1.336581 | 1.337200 | 68 |
| 69 | 1.340928 | 1.341551 | 1.342174 | 1.342798 | 1.343421 | 69 |
| 70 | 1.347174 | 1.347801 | 1.348427 | 1.349055 | 1.349682 | 70 |
| 71 | 1.353456 | 1.354087 | 1.354717 | 1.355349 | 1.355980 | 71 |
| 72 | 1.359778 | 1.360413 | 1.361047 | 1.361682 | 1.362317 | 72 |
| 73 | 1.366139 | 1.366777 | 1.367415 | 1.368054 | 1.368693 | 73 |
| 74 | 1.372536 | 1.373178 | 1.373820 | 1.374463 | 1.375105 | 74 |
| 75 | 1.378971 | 1.379617 | 1.380262 | 1.380909 | 1.381555 | 75 |
| 76 | 1.385446 | 1.386096 | 1.386745 | 1.387396 | 1.388045 | 76 |
| 77 | 1.391956 | 1.392610 | 1.393263 | 1.393917 | 1.394571 | 77 |
| 78 | 1.398505 | 1.399162 | 1.399819 | 1.400477 | 1.401134 | 78 |
| 79 | 1.405091 | 1.405752 | 1.406412 | 1.407074 | 1.407735 | 79 |
| 80 | 1.411715 | 1.412380 | 1.413044 | 1.413709 | 1.414374 | 80 |
| 81 | 1.418374 | 1.419043 | 1.419711 | 1.420380 | 1.421049 | 81 |
| 82 | 1.425072 | 1.425744 | 1.426416 | 1.427089 | 1.427761 | 82 |
| 83 | 1.431807 | 1.432483 | 1.433158 | 1.433835 | 1.434511 | 83 |
| 84 | 1.438579 | 1.439259 | 1.439938 | 1.440619 | 1.441299 | 84 |
| 85 | 1.445388 | 1.446071 | 1.446754 | 1.447438 | 1.448121 | 85 |
| 86 | 1.452232 | 1.452919 | 1.453605 | 1.454292 | 1.454980 | 86 |
| 87 | 1.459114 | 1.459805 | 1.460495 | 1.461186 | 1.461877 | 87 |
| 88 | 1.466032 | 1.466726 | 1.467420 | 1.468115 | 1.468810 | 88 |
| 89 | 1.472986 | 1.473684 | 1.474381 | 1.475080 | 1.475779 | 89 |
| 90 | 1.479976 | 1.480677 | 1.481378 | 1.482080 | 1.482782 | 90 |
| 91 | 1.487002 | 1.487707 | 1.488411 | 1.489117 | 1.489823 | 91 |
| 92 | 1.494063 | 1.494771 | 1.495479 | 1.496188 | 1.496897 | 92 |
| 93 | 1.501158 | 1.501870 | 1.502582 | 1.503293 | 1.504006 | 93 |
| 94 | 1.508289 | 1.509004 | 1.509720 | 1.510435 | 1.511151 | 94 |
| 95 | 1.515455 | 1.516174 | 1.516893 | 1.517612 | 1.518332 | 95 |
| 96 | 1.522656 | 1.523378 | 1.524100 | 1.524823 | 1.525546 | 96 |
| 97 | 1.529891 | 1.530616 | 1.531342 | 1.532068 | 1.532794 | 97 |
| 98 | 1.537161 | 1.537889 | 1.538618 | 1.539347 | 1.540076 | 98 |
| 99 | 1.544462 | 1.545194 | 1.545926 | 1.546659 | 1.547392 | 99 |
| 100 | 1.551800 | | | | | 100 |

*Densities of solutions of cane sugar at 20°C.*—Continued. 5

| PER CENT SUGAR | TENTHS OF PER CENT | | | | | PER CENT SUGAR |
|---|---|---|---|---|---|---|
| | 5 | 6 | 7 | 8 | 9 | |
| 50 | 1.232322 | 1.232874 | 1.233426 | 1.233979 | 1.234532 | 50 |
| 51 | 1.237859 | 1.238414 | 1.238970 | 1.239527 | 1.240084 | 51 |
| 52 | 1.243433 | 1.243992 | 1.244552 | 1.245113 | 1.245673 | 52 |
| 53 | 1.249046 | 1.249609 | 1.250172 | 1.250737 | 1.251301 | 53 |
| 54 | 1.254697 | 1.255264 | 1.255831 | 1.256400 | 1.256967 | 54 |
| 55 | 1.260385 | 1.260955 | 1.261527 | 1.262099 | 1.262671 | 55 |
| 56 | 1.266112 | 1.266686 | 1.267261 | 1.267837 | 1.268413 | 56 |
| 57 | 1.271877 | 1.272455 | 1.273035 | 1.273614 | 1.274194 | 57 |
| 58 | 1.277680 | 1.278262 | 1.278844 | 1.279428 | 1.280011 | 58 |
| 59 | 1.283521 | 1.284107 | 1.284694 | 1.285281 | 1.285869 | 59 |
| 60 | 1.289401 | 1.289991 | 1.290581 | 1.291172 | 1.291763 | 60 |
| 61 | 1.295318 | 1.295911 | 1.296506 | 1.297100 | 1.297696 | 61 |
| 62 | 1.301274 | 1.301871 | 1.302470 | 1.303068 | 1.303668 | 62 |
| 63 | 1.307271 | 1.307872 | 1.308475 | 1.309077 | 1.309680 | 63 |
| 64 | 1.313304 | 1.313909 | 1.314515 | 1.315121 | 1.315728 | 64 |
| 65 | 1.319374 | 1.319983 | 1.320593 | 1.321203 | 1.321814 | 65 |
| 66 | 1.325484 | 1.326097 | 1.326711 | 1.327325 | 1.327940 | 66 |
| 67 | 1.331633 | 1.332250 | 1.332868 | 1.333485 | 1.334103 | 67 |
| 68 | 1.337821 | 1.338441 | 1.339063 | 1.339684 | 1.340306 | 68 |
| 69 | 1.344046 | 1.344671 | 1.345296 | 1.345922 | 1.346547 | 69 |
| 70 | 1.350311 | 1.350939 | 1.351568 | 1.352197 | 1.352827 | 70 |
| 71 | 1.356612 | 1.357245 | 1.357877 | 1.358511 | 1.359144 | 71 |
| 72 | 1.362953 | 1.363590 | 1.364226 | 1.364864 | 1.365501 | 72 |
| 73 | 1.369333 | 1.369973 | 1.370613 | 1.371254 | 1.371894 | 73 |
| 74 | 1.375749 | 1.376392 | 1.377036 | 1.377680 | 1.378326 | 74 |
| 75 | 1.382203 | 1.382851 | 1.383499 | 1.384148 | 1.384796 | 75 |
| 76 | 1.388696 | 1.389347 | 1.389999 | 1.390651 | 1.391303 | 76 |
| 77 | 1.395226 | 1.395881 | 1.396536 | 1.397192 | 1.397848 | 77 |
| 78 | 1.401793 | 1.402452 | 1.403111 | 1.403771 | 1.404430 | 78 |
| 79 | 1.408398 | 1.409061 | 1.409723 | 1.410387 | 1.411051 | 79 |
| 80 | 1.415040 | 1.415706 | 1.416373 | 1.417039 | 1.417707 | 80 |
| 81 | 1.421719 | 1.422390 | 1.423059 | 1.423730 | 1.424400 | 81 |
| 82 | 1.428435 | 1.429109 | 1.429782 | 1.430457 | 1.431131 | 82 |
| 83 | 1.435188 | 1.435866 | 1.436543 | 1.437222 | 1.437900 | 83 |
| 84 | 1.441980 | 1.442661 | 1.443342 | 1.444024 | 1.444705 | 84 |
| 85 | 1.448806 | 1.449491 | 1.450175 | 1.450860 | 1.451545 | 85 |
| 86 | 1.455668 | 1.456357 | 1.457045 | 1.457735 | 1.458424 | 86 |
| 87 | 1.462568 | 1.463260 | 1.463953 | 1.464645 | 1.465338 | 87 |
| 88 | 1.469504 | 1.470200 | 1.470896 | 1.471592 | 1.472289 | 88 |
| 89 | 1.476477 | 1.477176 | 1.477876 | 1.478575 | 1.479275 | 89 |
| 90 | 1.483484 | 1.484187 | 1.484890 | 1.485593 | 1.486297 | 90 |
| 91 | 1.490528 | 1.491234 | 1.491941 | 1.492647 | 1.493355 | 91 |
| 92 | 1.497606 | 1.498316 | 1.499026 | 1.499736 | 1.500447 | 92 |
| 93 | 1.504719 | 1.505432 | 1.506146 | 1.506859 | 1.507574 | 93 |
| 94 | 1.511868 | 1.512585 | 1.513302 | 1.514019 | 1.514737 | 94 |
| 95 | 1.519051 | 1.519771 | 1.520492 | 1.521212 | 1.521934 | 95 |
| 96 | 1.526269 | 1.526993 | 1.527717 | 1.528441 | 1.529166 | 96 |
| 97 | 1.533521 | 1.534248 | 1.534976 | 1.535704 | 1.536432 | 97 |
| 98 | 1.540806 | 1.541536 | 1.542267 | 1.542998 | 1.543730 | 98 |
| 99 | 1.548127 | 1.548861 | 1.549595 | 1.550329 | 1.551064 | 99 |
| 100 | | | | | | 100 |

6 *Schönrock's table for determining water in sugar solutions by means of the Abbé refractometer.*

| REFRACTIVE INDEX AT 20° | WATER, PER CENT | REFRACTIVE INDEX AT 20° | WATER, PER CENT | REFRACTIVE INDEX AT 20° | WATER, PER CENT | REFRACTIVE INDEX AT 20° | WATER, PER CENT | REFRACTIVE INDEX AT 20° | WATER, PER CENT |
|---|---|---|---|---|---|---|---|---|---|
| 1.3330 | 100.0 | 1.3464 | 91.0 | 1.3606 | 82.0 | 1.3758 | 73.0 | 1.3920 | 64.0 |
| 1.3333 | 99.8 | 1.3467 | 90.8 | 1.3609 | 81.8 | 1.3761 | 72.8 | 1.3924 | 63.8 |
| 1.3336 | 99.6 | 1.3470 | 90.6 | 1.3612 | 81.6 | 1.3765 | 72.6 | 1.3928 | 63.6 |
| 1.3338 | 99.4 | 1.3473 | 90.4 | 1.3616 | 81.4 | 1.3768 | 72.4 | 1.3931 | 63.4 |
| 1.3341 | 99.2 | 1.3476 | 90.2 | 1.3619 | 81.2 | 1.3772 | 72.2 | 1.3935 | 63.2 |
| 1.3344 | 99.0 | 1.3479 | 90.0 | 1.3622 | 81.0 | 1.3775 | 72.0 | 1.3939 | 63.0 |
| 1.3347 | 98.8 | 1.3482 | 89.8 | 1.3625 | 80.8 | 1.3779 | 71.8 | 1.3943 | 62.8 |
| 1.3350 | 98.6 | 1.3485 | 89.6 | 1.3629 | 80.6 | 1.3782 | 71.6 | 1.3947 | 62.6 |
| 1.3353 | 98.4 | 1.3488 | 89.4 | 1.3632 | 80.4 | 1.3786 | 71.4 | 1.3950 | 62.4 |
| 1.3356 | 98.2 | 1.3491 | 89.2 | 1.3636 | 80.2 | 1.3789 | 71.2 | 1.3954 | 62.2 |
| 1.3359 | 98.0 | 1.3494 | 89.0 | 1.3939 | 80.0 | 1.3793 | 71.0 | 1.3958 | 62.0 |
| 1.3362 | 97.8 | 1.3497 | 88.8 | 1.3642 | 79.8 | 1.3797 | 70.8 | 1.3962 | 61.8 |
| 1.3365 | 97.6 | 1.3500 | 88.6 | 1.3645 | 79.6 | 1.3800 | 70.6 | 1.3966 | 61.6 |
| 1.3368 | 97.4 | 1.3504 | 88.4 | 1.3649 | 79.4 | 1.3804 | 70.4 | 1.3970 | 61.4 |
| 1.3371 | 97.2 | 1.3507 | 88.2 | 1.3652 | 79.2 | 1.3807 | 70.2 | 1.3974 | 61.2 |
| 1.3374 | 97.0 | 1.3510 | 88.0 | 1.3655 | 79.0 | 1.3811 | 70.0 | 1.3978 | 61.0 |
| 1.3377 | 96.8 | 1.3513 | 87.8 | 1.3658 | 78.8 | 1.3815 | 69.8 | 1.3982 | 60.8 |
| 1.3380 | 96.6 | 1.3516 | 87.6 | 1.3662 | 78.6 | 1.3818 | 69.6 | 1.3986 | 60.6 |
| 1.3382 | 96.4 | 1.3520 | 87.4 | 1.3665 | 78.4 | 1.3822 | 69.4 | 1.3989 | 60.4 |
| 1.3385 | 96.2 | 1.3523 | 87.2 | 1.3669 | 78.2 | 1.3825 | 69.2 | 1.3993 | 60.2 |
| 1.3388 | 96.0 | 1.3526 | 87.0 | 1.3672 | 78.0 | 1.3829 | 69.0 | 1.3997 | 60.0 |
| 1.3391 | 95.8 | 1.3529 | 86.8 | 1.3675 | 77.8 | 1.3833 | 68.8 | 1.4001 | 59.8 |
| 1.3394 | 95.6 | 1.3532 | 86.6 | 1.3679 | 77.6 | 1.3836 | 68.6 | 1.4005 | 59.6 |
| 1.3397 | 95.4 | 1.3535 | 86.4 | 1.3682 | 77.4 | 1.3840 | 68.4 | 1.4008 | 59.4 |
| 1.3400 | 95.2 | 1.3538 | 86.2 | 1.3686 | 77.2 | 1.3843 | 68.2 | 1.4012 | 59.2 |
| 1.3403 | 95.0 | 1.3541 | 86.0 | 1.3689 | 77.0 | 1.3847 | 68.0 | 1.4016 | 59.0 |
| 1.3406 | 94.8 | 1.3544 | 85.8 | 1.3692 | 76.8 | 1.3851 | 67.8 | 1.4020 | 58.8 |
| 1.3409 | 94.6 | 1.3547 | 85.6 | 1.3696 | 76.6 | 1.3854 | 67.6 | 1.4024 | 58.6 |
| 1.3412 | 94.4 | 1.3551 | 85.4 | 1.3699 | 76.4 | 1.3858 | 67.4 | 1.4028 | 58.4 |
| 1.3415 | 94.2 | 1.3554 | 85.2 | 1.3703 | 76.2 | 1.3861 | 67.2 | 1.4032 | 58.2 |
| 1.3418 | 94.0 | 1.3557 | 85.0 | 1.3706 | 76.0 | 1.3865 | 67.0 | 1.4036 | 58.0 |
| 1.3421 | 93.8 | 1.3560 | 84.8 | 1.3709 | 75.8 | 1.3869 | 66.8 | 1.4040 | 57.8 |
| 1.3424 | 93.6 | 1.3563 | 84.6 | 1.3713 | 75.6 | 1.3872 | 66.6 | 1.4044 | 57.6 |
| 1.3427 | 93.4 | 1.3567 | 84.4 | 1.3716 | 75.4 | 1.3876 | 66.4 | 1.4048 | 57.4 |
| 1.3430 | 93.2 | 1.3570 | 84.2 | 1.3720 | 75.2 | 1.3879 | 66.2 | 1.4052 | 57.2 |
| 1.3433 | 93.0 | 1.3573 | 84.0 | 1.3723 | 75.0 | 1.3883 | 66.0 | 1.4056 | 57.0 |
| 1.3436 | 92.8 | 1.3576 | 83.8 | 1.3726 | 74.8 | 1.3887 | 65.8 | 1.4060 | 56.8 |
| 1.3439 | 92.6 | 1.3580 | 83.6 | 1.3730 | 74.6 | 1.3891 | 65.6 | 1.4064 | 56.6 |
| 1.3442 | 92.4 | 1.3583 | 83.4 | 1.3733 | 74.4 | 1.3894 | 65.4 | 1.4068 | 56.4 |
| 1.3445 | 92.2 | 1.3587 | 83.2 | 1.3737 | 74.2 | 1.3898 | 65.2 | 1.4072 | 56.2 |
| 1.3448 | 92.0 | 1.3590 | 83.0 | 1.3740 | 74.0 | 1.3902 | 65.0 | 1.4076 | 56.0 |
| 1.3451 | 91.8 | 1.3593 | 82.8 | 1.3744 | 73.8 | 1.3906 | 64.8 | 1.4080 | 55.8 |
| 1.3454 | 91.6 | 1.3596 | 82.6 | 1.3747 | 73.6 | 1.3909 | 64.6 | 1.4084 | 55.6 |
| 1.3458 | 91.4 | 1.3600 | 82.4 | 1.3751 | 73.4 | 1.3913 | 64.4 | 1.4088 | 55.4 |
| 1.3461 | 91.2 | 1.3603 | 82.2 | 1.3754 | 73.2 | 1.3916 | 64.2 | 1.4092 | 55.2 |

### *Schönrock's table.*—Continued. 6

| REFRACTIVE INDEX AT 20° | WATER, PER CENT | REFRACTIVE INDEX AT 20° | WATER, PER CENT | REFRACTIVE INDEX AT 20° | WATER, PER CENT | REFRACTIVE INDEX AT 20° | WATER, PER CENT | REFRACTIVE INDEX AT 20° | WATER, PER CENT |
|---|---|---|---|---|---|---|---|---|---|
| 1.4096 | 55.0 | 1.4264 | 47.0 | 1.4441 | 39.0 | 1.4623 | 31.2 | 1.4820 | 23.2 |
| 1.4100 | 54.8 | 1.4268 | 46.8 | 1.4446 | 38.8 | 1.4628 | 31.0 | 1.4825 | 23.0 |
| 1.4104 | 54.6 | 1.4272 | 46.6 | 1.4450 | 38.6 | 1.4632 | 30.8 | 1.4830 | 22.8 |
| 1.4109 | 54.4 | 1.4277 | 46.4 | 1.4455 | 38.4 | 1.4637 | 30.6 | 1.4835 | 22.6 |
| 1.4113 | 54.2 | 1.4281 | 46.2 | 1.4459 | 38.2 | 1.4642 | 30.4 | 1.4840 | 22.4 |
| 1.4117 | 54.0 | 1.4285 | 46.0 | 1.4464 | 38.0 | 1.4646 | 30.2 | 1.4845 | 22.2 |
| 1.4121 | 53.8 | 1.4289 | 45.8 | 1.4468 | 37.8 | 1.4651 | 30.0 | 1.4850 | 22.0 |
| 1.4125 | 53.6 | 1.4294 | 45.6 | 1.4473 | 37.6 | 1.4656 | 29.8 | 1.4855 | 21.8 |
| 1.4129 | 53.4 | 1.4298 | 45.4 | 1.4477 | 37.4 | 1.4661 | 29.6 | 1.4860 | 21.6 |
| 1.4133 | 53.2 | 1.4303 | 45.2 | 1.4482 | 37.2 | 1.4666 | 29.4 | 1.4865 | 21.4 |
| 1.4137 | 53.0 | 1.4307 | 45.0 | 1.4486 | 37.0 | 1.4671 | 29.2 | 1.4871 | 21.2 |
| 1.4141 | 52.8 | 1.4311 | 44.8 | 1.4491 | 36.8 | 1.4676 | 29.0 | 1.4876 | 21.0 |
| 1.4145 | 52.6 | 1.4316 | 44.6 | 1.4495 | 36.6 | 1.4681 | 28.8 | 1.4881 | 20.8 |
| 1.4150 | 52.4 | 1.4320 | 44.4 | 1.4500 | 36.4 | 1.4685 | 28.6 | 1.4886 | 20.6 |
| 1.4154 | 52.2 | 1.4325 | 44.2 | 1.4504 | 36.2 | 1.4690 | 28.4 | 1.4891 | 20.4 |
| 1.4158 | 52.0 | 1.4329 | 44.0 | 1.4509 | 36.0 | 1.4695 | 28.2 | 1.4896 | 20.2 |
| 1.4162 | 51.8 | 1.4333 | 43.8 | 1.4514 | 35.8 | 1.4700 | 28.0 | 1.4901 | 20.0 |
| 1.4166 | 51.6 | 1.4338 | 43.6 | 1.4518 | 35.6 | 1.4705 | 27.8 | 1.4906 | 19.8 |
| 1.4171 | 51.4 | 1.4342 | 43.4 | 1.4523 | 35.4 | 1.4710 | 27.6 | 1.4912 | 19.6 |
| 1.4175 | 51.2 | 1.4347 | 43.2 | 1.4527 | 35.2 | 1.4715 | 27.4 | 1.4917 | 19.4 |
| 1.4179 | 51.0 | 1.4351 | 43.0 | 1.4532 | 35.0 | 1.4720 | 27.2 | 1.4922 | 19.2 |
| 1.4183 | 50.8 | 1.4355 | 42.8 | 1.4537 | 34.8 | 1.4725 | 27.0 | 1.4927 | 19.0 |
| 1.4187 | 50.6 | 1.4360 | 42.6 | 1.4541 | 34.6 | 1.4730 | 26.8 | 1.4933 | 18.8 |
| 1.4192 | 50.4 | 1.4364 | 42.4 | 1.4546 | 34.4 | 1.4735 | 26.6 | 1.4938 | 18.6 |
| 1.4196 | 50.2 | 1.4369 | 42.2 | 1.4550 | 34.2 | 1.4740 | 26.4 | 1.4943 | 18.4 |
| 1.4200 | 50.0 | 1.4373 | 42.0 | 1.4555 | 34.0[1] | 1.4744 | 26.2 | 1.4949 | 18.2 |
| 1.4204 | 49.8 | 1.4378 | 41.8 | 1.4558 | 34.0 | 1.4749 | 26.0 | 1.4954 | 18.0 |
| 1.4208 | 49.6 | 1.4832 | 41.6 | 1.4563 | 33.8 | 1.4754 | 25.8 | 1.4959 | 17.8 |
| 1.4213 | 49.4 | 1.4387 | 41.4 | 1.4567 | 33.6 | 1.4759 | 25.6 | 1.4964 | 17.6 |
| 1.4217 | 49.2 | 1.4391 | 41.2 | 1.4572 | 33.4 | 1.4764 | 25.4 | 1.4970 | 17.4 |
| 1.4221 | 49.0 | 1.4396 | 41.0 | 1.4577 | 33.2 | 1.4769 | 25.2 | 1.4975 | 17.2 |
| 1.4225 | 48.8 | 1.4400 | 40.8 | 1.4581 | 33.0 | 1.4774 | 25.0 | 1.4980 | 17.0 |
| 1.4229 | 48.6 | 1.4405 | 40.6 | 1.4586 | 32.8 | 1.4779 | 24.8 | 1.4985 | 16.8 |
| 1.4234 | 48.4 | 1.4409 | 40.4 | 1.4591 | 32.6 | 1.4784 | 24.6 | 1.4991 | 16.6 |
| 1.4238 | 48.2 | 1.4414 | 40.2 | 1.4595 | 32.4 | 1.4789 | 24.4 | 1.4996 | 16.4 |
| 1.4242 | 48.0 | 1.4418 | 40.0 | 1.4600 | 32.2 | 1.4794 | 24.2 | 1.5001 | 16.2 |
| 1.4246 | 47.8 | 1.4423 | 39.8 | 1.4605 | 32.0 | 1.4799 | 24.0 | 1.5007 | 16.0 |
| 1.4251 | 47.6 | 1.4427 | 39.6 | 1.4609 | 31.8 | 1.4804 | 23.8 | 1.5012 | 15.8 |
| 1.4255 | 47.4 | 1.4432 | 39.4 | 1.4614 | 31.6 | 1.4810 | 23.6 | 1.5017 | 15.6 |
| 1.4260 | 47.2 | 1.4436 | 39.2 | 1.4619 | 31.4 | 1.4815 | 23.4 | 1.5022 | 15.4 |
|  |  |  |  |  |  |  |  | 1.5028 | 15.2 |
|  |  |  |  |  |  |  |  | 1.5033 | 15.0 |

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[1] The values of the refractive index from 34 per cent to 15 per cent are taken from Main's table, Inst. Sugar J. **9,** 481 (1907).

7 *Stanek's correction table for determining water in sugar solutions by means of the Abbé refractometer when readings are made at other temperatures than 20°C.*

| WATER, PER CENT | 95 | 90 | 85 | 80 | 70 | 60 | 50 | 40 | 30 | 25 | WATER, PER CENT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TEMPERATURE, °C. | TO BE ADDED TO THE PERCENTAGE OF WATER | | | | | | | | | | TEMPERATURE, °C. |
| 15 | 0.25 | 0.27 | 0.31 | 0.31 | 0.34 | 0.35 | 0.36 | 0.37 | 0.36 | 0.36 | 15 |
| 16 | 0.21 | 0.23 | 0.26 | 0.27 | 0.29 | 0.31 | 0.31 | 0.32 | 0.31 | 0.29 | 16 |
| 17 | 0.16 | 0.18 | 0.20 | 0.20 | 0.22 | 0.23 | 0.23 | 0.23 | 0.20 | 0.17 | 17 |
| 18 | 0.11 | 0.12 | 0.14 | 0.14 | 0.15 | 0.16 | 0.16 | 0.15 | 0.12 | 0.09 | 18 |
| 19 | 0.06 | 0.07 | 0.08 | 0.08 | 0.08 | 0.09 | 0.09 | 0.08 | 0.07 | 0.05 | 19 |
| TEMPERATURE, °C. | TO BE SUBTRACTED FROM THE PERCENTAGE OF WATER | | | | | | | | | | TEMPERATURE, °C |
| 21 | 0.06 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 21 |
| 22 | 0.12 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.15 | 0.14 | 0.14 | 0.14 | 22 |
| 23 | 0.18 | 0.20 | 0.20 | 0.21 | 0.21 | 0.21 | 0.23 | 0.21 | 0.22 | 0.22 | 23 |
| 24 | 0.24 | 0.26 | 0.26 | 0.27 | 0.28 | 0.28 | 0.30 | 0.28 | 0.29 | 0.29 | 24 |
| 25 | 0.30 | 0.32 | 0.32 | 0.34 | 0.36 | 0.36 | 0.38 | 0.36 | 0.36 | 0.37 | 25 |
| 26 | 0.36 | 0.39 | 0.39 | 0.41 | 0.43 | 0.43 | 0.46 | 0.44 | 0.43 | 0.44 | 26 |
| 27 | 0.43 | 0.46 | 0.46 | 0.48 | 0.50 | 0.51 | 0.55 | 0.52 | 0.50 | 0.51 | 27 |
| 28 | 0.50 | 0.53 | 0.53 | 0.55 | 0.58 | 0.59 | 0.63 | 0.60 | 0.57 | 0.59 | 28 |
| 29 | 0.57 | 0.60 | 0.61 | 0.62 | 0.66 | 0.67 | 0.71 | 0.68 | 0.65 | 0.67 | 29 |
| 30 | 0.64 | 0.67 | 0.70 | 0.71 | 0.74 | 0.75 | 0.80 | 0.76 | 0.73 | 0.75 | 30 |
| WATER, PER CENT | 95 | 90 | 85 | 80 | 70 | 60 | 50 | 40 | 30 | 25 | WATER, PER CENT |

*Hübener's table for determining percentages by weight of sucrose in sugar solutions from readings of the Zeiss immersion refractometer.* 8

| SCALE READING OF REFRACTOMETER | PER CENT SUCROSE | SCALE READING OF REFRACTOMETER | PER CENT SUCROSE | SCALE READING OF REFRACTOMETER | PER CENT SUCROSE | SCALE READING OF REFRACTOMETER | PER CENT SUCROSE | SCALE READING OF REFRACTOMETER | PER CENT SUCROSE | SCALE READING OF REFRACTOMETER | PER CENT SUCROSE | SCALE READING OF REFRACTOMETER | PER CENT SUCROSE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15.0 | 0.00 | 28.0 | .42 | 41.0 | .82 | 54.0 | .05 | 67.0 | .16 | 80.0 | .09 | 93.0 | .95 |
| .2 | 0.05 | .2 | .48 | .2 | .87 | .2 | .10 | .2 | .20 | .2 | .13 | .2 | 19.00 |
| .4 | 0.11 | .4 | .53 | .4 | .92 | .4 | .15 | .4 | .25 | .4 | .18 | .4 | .04 |
| .6 | 0.16 | .6 | .58 | .6 | .97 | .6 | .19 | .6 | .29 | .6 | .22 | .6 | .08 |
| .8 | 0.21 | .8 | .64 | .8 | 7.03 | .8 | .24 | .8 | .34 | .8 | .27 | .8 | .13 |
| 16.0 | 0.26 | 29.0 | .69 | 42.0 | .08 | 55.0 | .29 | 68.0 | .38 | 81.0 | .31 | 94.0 | .17 |
| .2 | 0.32 | .2 | .74 | .2 | .13 | .2 | .34 | .2 | .43 | .2 | .35 | .2 | .21 |
| .4 | 0.37 | .4 | .79 | .4 | .18 | .4 | .39 | .4 | .48 | .4 | .40 | .4 | .25 |
| .6 | 0.42 | .6 | .84 | .6 | .23 | .6 | .44 | .6 | .52 | .6 | .44 | .6 | .29 |
| .8 | 0.48 | .8 | .90 | .8 | .28 | .8 | .49 | .8 | .57 | .8 | .49 | .8 | .34 |
| 17.0 | 0.53 | 30.0 | .95 | 43.0 | .33 | 56.0 | .53 | 69.0 | .61 | 82.0 | .54 | 95.0 | .38 |
| .2 | 0.58 | .2 | 4.00 | .2 | .39 | .2 | .58 | .2 | .66 | .2 | .59 | .2 | .42 |
| .4 | 0.64 | .4 | .05 | .4 | .43 | .4 | .63 | .4 | .70 | .4 | .63 | .4 | .47 |
| .6 | 0.69 | .6 | .11 | .6 | .49 | .6 | .68 | .6 | .75 | .6 | .68 | .6 | .51 |
| .8 | 0.74 | .8 | .16 | .8 | .54 | .8 | .73 | .8 | .79 | .8 | .72 | .8 | .55 |
| 18.0 | 0.79 | 31.0 | .21 | 44.0 | .59 | 57.0 | .78 | 70.0 | .84 | 83.0 | .76 | 96.0 | .59 |
| .2 | 0.84 | .2 | .26 | .2 | .64 | .2 | .83 | .2 | .89 | .2 | .81 | .2 | .63 |
| .4 | 0.90 | .4 | .32 | .4 | .69 | .4 | .88 | .4 | .94 | .4 | .85 | .4 | .68 |
| .6 | 0.95 | .6 | .37 | .6 | .74 | .6 | .92 | .6 | .98 | .6 | .90 | .6 | .72 |
| .8 | 1.00 | .8 | .42 | .8 | .79 | .8 | .97 | .8 | 14.03 | .8 | .95 | .8 | .76 |
| 19.0 | .05 | 32.0 | .48 | 45.0 | .84 | 58.0 | 11.03 | 71.0 | .07 | 84.0 | 17.00 | 97.0 | .80 |
| .2 | .11 | .2 | .53 | .2 | .90 | .2 | .07 | .2 | .11 | .2 | .04 | .2 | .85 |
| .4 | .16 | .4 | .58 | .4 | .95 | .4 | .12 | .4 | .16 | .4 | .09 | .4 | .89 |
| .6 | .21 | .6 | .64 | .6 | 8.00 | .6 | .17 | .6 | .20 | .6 | .13 | .6 | .93 |
| .8 | .26 | .8 | .69 | .8 | .05 | .8 | .22 | .8 | .25 | .8 | .18 | .8 | .97 |
| 20.0 | .32 | 33.0 | .74 | 46.0 | .10 | 59.0 | .27 | 72.0 | .29 | 85.0 | .22 | 98.0 | 20.02 |
| .2 | .37 | .2 | .79 | .2 | .15 | .2 | .32 | .2 | .34 | .2 | .27 | .2 | .06 |
| .4 | .42 | .4 | .84 | .4 | .19 | .4 | .36 | .4 | .38 | .4 | .31 | .4 | .10 |
| .6 | .48 | .6 | .90 | .6 | .24 | .6 | .41 | .6 | .43 | .6 | .35 | .6 | .15 |
| .8 | .53 | .8 | .95 | .8 | .29 | .8 | .46 | .8 | .48 | .8 | .40 | .8 | .19 |
| 21.0 | .58 | 34.0 | 5.00 | 47.0 | .34 | 60.0 | .51 | 73.0 | .52 | 86.0 | .44 | 99.0 | .23 |
| .2 | .64 | .2 | .05 | .2 | .39 | .2 | .56 | .2 | .57 | .2 | .49 | .2 | .27 |
| .4 | .69 | .4 | .11 | .4 | .44 | .4 | .60 | .4 | .61 | .4 | .53 | .4 | .31 |
| .6 | .74 | .6 | .16 | .6 | .49 | .6 | .66 | .6 | .66 | .6 | .58 | .6 | .36 |
| .8 | .79 | .8 | .21 | .8 | .53 | .8 | .70 | .8 | .70 | .8 | .62 | .8 | .40 |
| 22.0 | .84 | 35.0 | .26 | 48.0 | .58 | 61.0 | .75 | 74.0 | .75 | 87.0 | .66 | 100.0 | .44 |
| .2 | .90 | .2 | .32 | .2 | .63 | .2 | .80 | .2 | .79 | .2 | .71 | .2 | .49 |
| .4 | .95 | .4 | .37 | .4 | .68 | .4 | .85 | .4 | .84 | .4 | .75 | .4 | .53 |
| .6 | 2.00 | .6 | .42 | .6 | .73 | .6 | .90 | .6 | .89 | .6 | .79 | .6 | .57 |
| .8 | .05 | .8 | .48 | .8 | .78 | .8 | .95 | .8 | .94 | .8 | .84 | .8 | .61 |
| 23.0 | .11 | 36.0 | .53 | 49.0 | .83 | 62.0 | 12.00 | 75.0 | .98 | 88.0 | .89 | 101.0 | .66 |
| .2 | .16 | .2 | .58 | .2 | .88 | .2 | .05 | .2 | 15.03 | .2 | .93 | .2 | .70 |
| .4 | .21 | .4 | .64 | .4 | .92 | .4 | .09 | .4 | .07 | .4 | .98 | .4 | .74 |
| .6 | .26 | .6 | .69 | .6 | .97 | .6 | .14 | .6 | .11 | .6 | 18.02 | .6 | .78 |
| .8 | .32 | .8 | .74 | .8 | 9.03 | .8 | .18 | .8 | .16 | .8 | .06 | .8 | .82 |
| 24.0 | .37 | 37.0 | .79 | 50.0 | .07 | 63.0 | .23 | 76.0 | .20 | 89.0 | .10 | 102.0 | .87 |
| .2 | .42 | .2 | .84 | .2 | .12 | .2 | .28 | .2 | .24 | .2 | .15 | .2 | .91 |
| .4 | .48 | .4 | .90 | .4 | .17 | .4 | .32 | .4 | .28 | .4 | .19 | .4 | .95 |
| .6 | .53 | .6 | .95 | .6 | .22 | .6 | .37 | .6 | .32 | .6 | .23 | .6 | 21.00 |
| .8 | .58 | .8 | 6.00 | .8 | .27 | .8 | .42 | .8 | .36 | .8 | .27 | .8 | .04 |
| 25.0 | .64 | 38.0 | .05 | 51.0 | .32 | 64.0 | .46 | 77.0 | .40 | 90.0 | .31 | 103.0 | .08 |
| .2 | .69 | .2 | .10 | .2 | .36 | .2 | .51 | .2 | .44 | .2 | .36 | .2 | .13 |
| .4 | .74 | .4 | .15 | .4 | .41 | .4 | .56 | .4 | .49 | .4 | .40 | .4 | .17 |
| .6 | .79 | .6 | .20 | .6 | .46 | .6 | .60 | .6 | .54 | .6 | .44 | .6 | .21 |
| .8 | .84 | .8 | .26 | .8 | .51 | .8 | .65 | .8 | .59 | .8 | .49 | .8 | .25 |
| 26.0 | .90 | 39.0 | .31 | 52.0 | .56 | 65.0 | .69 | 78.0 | .63 | 91.0 | .53 | 104.0 | .29 |
| .2 | .95 | .2 | .36 | .2 | .60 | .2 | .74 | .2 | .68 | .2 | .57 | .2 | .34 |
| .4 | 3.00 | .4 | .41 | .4 | .66 | .4 | .79 | .4 | .72 | .4 | .61 | .4 | .38 |
| .6 | .05 | .6 | .46 | .6 | .70 | .6 | .83 | .6 | .76 | .6 | .66 | .6 | .42 |
| .8 | .11 | .8 | .51 | .8 | .75 | .8 | .88 | .8 | .81 | .8 | .70 | .8 | .47 |
| 27.0 | .16 | 40.0 | .56 | 53.0 | .80 | 66.0 | .93 | 79.0 | .85 | 92.0 | .74 | 105.0 | .51 |
| .2 | .21 | .2 | .61 | .2 | .85 | .2 | .97 | .2 | .90 | .2 | .78 | .2 | .55 |
| .4 | .26 | .4 | .66 | .4 | .90 | .4 | 13.03 | .4 | .95 | .4 | .82 | .4 | .59 |
| .6 | .32 | .6 | .72 | .6 | .95 | .6 | .07 | .6 | 16.00 | .6 | .87 | .6 | .63 |
| .8 | .37 | .8 | .77 | .8 | 10.00 | .8 | .11 | .8 | .05 | .8 | .91 | .8 | .68 |
| ... | ...... | ...... | ...... | ...... | ...... | ...... | ...... | ...... | ...... | ...... | ...... | 106.0 | 21.71 |

### 9 *Munson and Walker's table*[1] *for calculating dextrose, invert sugar alone, invert sugar in the presence of sucrose (0.4 gram and 2 grams total sugar), lactose, lactose and sucrose (2 mixtures), and maltose (crystallized).*

(Expressed in milligrams.)

| CUPROUS OXIDE ($Cu_2O$) | COPPER (Cu) | DEXTROSE (*d*-GLUCOSE) | INVERT SUGAR | INVERT SUGAR AND SUCROSE | | LACTOSE | LACTOSE AND SUCROSE | | MALTOSE | CUPROUS OXIDE ($Cu_2O$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar | 2 grams total sugar | $C_{12}H_{22}O_{11}+H_2O$ | 1 lactose, 4 sucrose | 1 lactose, 12 sucrose | $C_{12}H_{22}O_{11}+H_2O$ | |
| 10 | 8.9 | 4.0 | 4.5 | 1.6 | ...... | 6.3 | 6.1 | ...... | 6.2 | 10 |
| 12 | 10.7 | 4.9 | 5.4 | 2.5 | ...... | 7.5 | 7.3 | ...... | 7.9 | 12 |
| 14 | 12.4 | 5.7 | 6.3 | 3.4 | ...... | 8.8 | 8.5 | ...... | 9.5 | 14 |
| 16 | 14.2 | 6.6 | 7.2 | 4.3 | ...... | 10.0 | 9.7 | ...... | 11.2 | 16 |
| 18 | 16.0 | 7.5 | 8.1 | 5.2 | ...... | 11.3 | 10.9 | ...... | 12.9 | 18 |
| 20 | 17.8 | 8.3 | 8.9 | 6.1 | ...... | 12.5 | 12.1 | ...... | 14.6 | 20 |
| 22 | 19.5 | 9.2 | 9.8 | 7.0 | ...... | 13.8 | 13.3 | ...... | 16.2 | 22 |
| 24 | 21.3 | 10.0 | 10.7 | 7.9 | ...... | 15.0 | 14.5 | ...... | 17.9 | 24 |
| 26 | 23.1 | 10.9 | 11.6 | 8.8 | ...... | 16.3 | 15.8 | ...... | 19.6 | 26 |
| 28 | 24.9 | 11.8 | 12.5 | 9.7 | ...... | 17.6 | 17.0 | ...... | 21.2 | 28 |
| 30 | 26.6 | 12.6 | 13.4 | 10.7 | 4.3 | 18.8 | 18.2 | ...... | 22.9 | 30 |
| 32 | 28.4 | 13.5 | 14.3 | 11.6 | 5.2 | 20.1 | 19.4 | ...... | 24.6 | 32 |
| 34 | 30.2 | 14.3 | 15.2 | 12.5 | 6.1 | 21.4 | 20.7 | ...... | 26.2 | 34 |
| 36 | 32.0 | 15.2 | 16.1 | 13.4 | 7.0 | 22.8 | 22.0 | ...... | 27.9 | 36 |
| 38 | 33.8 | 16.1 | 16.9 | 14.3 | 7.9 | 24.2 | 23.3 | ...... | 29.6 | 38 |
| 40 | 35.5 | 16.9 | 17.8 | 15.2 | 8.8 | 25.5 | 24.7 | ...... | 31.3 | 40 |
| 42 | 37.3 | 17.8 | 18.7 | 16.1 | 9.7 | 29.6 | 26.0 | ...... | 32.9 | 42 |
| 44 | 39.1 | 18.7 | 19.6 | 17.0 | 10.7 | 28.3 | 27.3 | ...... | 34.6 | 44 |
| 46 | 40.9 | 19.6 | 20.5 | 17.9 | 11.6 | 29.6 | 28.6 | ...... | 36.3 | 46 |
| 48 | 42.6 | 20.4 | 21.4 | 18.8 | 12.5 | 31.0 | 30.0 | ...... | 37.9 | 48 |
| 50 | 44.4 | 21.3 | 22.3 | 19.7 | 13.4 | 32.3 | 31.3 | ...... | 39.6 | 50 |
| 52 | 46.2 | 22.2 | 23.2 | 20.7 | 14.3 | 33.7 | 32.6 | ...... | 41.3 | 52 |
| 54 | 48.0 | 23.0 | 24.1 | 21.6 | 15.2 | 35.1 | 34.0 | ...... | 42.9 | 54 |
| 56 | 49.7 | 23.9 | 25.0 | 22.5 | 16.2 | 36.4 | 35.3 | ...... | 44.6 | 56 |
| 58 | 51.5 | 24.8 | 25.9 | 23.4 | 17.1 | 37.8 | 36.6 | ...... | 46.3 | 58 |
| 60 | 53.3 | 25.6 | 26.8 | 24.3 | 18.0 | 39.2 | 37.9 | ...... | 48.0 | 60 |
| 62 | 55.1 | 26.5 | 27.7 | 25.2 | 18.9 | 40.5 | 39.3 | ...... | 49.6 | 62 |
| 64 | 56.8 | 27.4 | 28.6 | 26.2 | 19.8 | 41.9 | 40.6 | ...... | 51.3 | 64 |
| 66 | 58.6 | 28.3 | 29.5 | 27.1 | 20.8 | 43.3 | 41.9 | ...... | 53.0 | 66 |
| 68 | 60.4 | 29.2 | 30.4 | 28.0 | 21.7 | 44.7 | 43.3 | 40.7 | 54.6 | 68 |
| 70 | 62.2 | 30.0 | 31.3 | 28.9 | 22.6 | 46.0 | 44.6 | 41.9 | 56.3 | 70 |
| 72 | 64.0 | 30.9 | 32.3 | 29.8 | 23.5 | 47.4 | 45.9 | 43.1 | 58.0 | 72 |
| 74 | 65.7 | 31.8 | 33.2 | 30.8 | 24.5 | 48.8 | 47.3 | 44.2 | 59.6 | 74 |
| 76 | 67.5 | 32.7 | 34.1 | 31.7 | 25.4 | 50.1 | 48.6 | 45.4 | 61.3 | 76 |
| 78 | 69.3 | 33.6 | 35.0 | 32.6 | 26.3 | 51.5 | 49.9 | 46.6 | 63.0 | 78 |
| 80 | 71.1 | 34.4 | 35.9 | 33.5 | 27.3 | 52.9 | 51.3 | 47.8 | 64.6 | 80 |
| 82 | 72.8 | 35.3 | 36.8 | 34.5 | 28.2 | 54.2 | 52.6 | 49.0 | 66.3 | 82 |
| 84 | 74.6 | 36.2 | 37.7 | 35.4 | 29.1 | 55.6 | 53.9 | 50.1 | 68.0 | 84 |
| 86 | 76.4 | 37.1 | 38.6 | 36.3 | 30.0 | 57.0 | 55.3 | 51.3 | 69.7 | 86 |
| 88 | 78.2 | 38.0 | 39.5 | 37.2 | 31.0 | 58.4 | 56.6 | 52.5 | 71.3 | 88 |

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[1] U. S. Bur. Standards Circ. 44, p. 321. The columns headed "Lactose" and "Lactose and Sucrose" are taken from "Methods of Sugar Analysis and Allied Determinations" by Arthur Given.

### *Munson and Walker's table.*—Continued.

(Expressed in milligrams.)

| CUPROUS OXIDE ($Cu_2O$) | COPPER (Cu) | DEXTROSE (*d*-GLUCOSE) | INVERT SUGAR | INVERT SUGAR AND SUCROSE | | LACTOSE | LACTOSE AND SUCROSE | | MALTOSE | CUPROUS OXIDE ($Cu_2O$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar | 2 grams total sugar | $C_{12}H_{22}O_{11}+H_2O$ | 1 lactose, 4 sucrose | 1 lactose, 12 sucrose | $C_{12}H_{22}O_{11}+H_2O$ | |
| 90 | 79.9 | 38.9 | 40.4 | 38.2 | 31.9 | 59.7 | 57.9 | 53.7 | 73.0 | 90 |
| 92 | 81.7 | 39.8 | 41.4 | 39.1 | 32.8 | 61.1 | 59.3 | 54.9 | 74.7 | 92 |
| 94 | 83.5 | 40.6 | 42.3 | 40.0 | 33.8 | 62.5 | 60.6 | 56.0 | 76.3 | 94 |
| 96 | 85.3 | 41.5 | 43.2 | 41.0 | 34.7 | 63.8 | 61.9 | 57.2 | 78.0 | 96 |
| 98 | 87.1 | 42.4 | 44.1 | 41.9 | 35.6 | 65.2 | 63.3 | 58.4 | 79.7 | 98 |
| 100 | 88.8 | 43.3 | 45.0 | 42.8 | 36.6 | 66.6 | 64.6 | 59.6 | 81.3 | 100 |
| 102 | 90.6 | 44.2 | 46.0 | 43.8 | 37.5 | 68.0 | 66.0 | 60.8 | 83.0 | 102 |
| 104 | 92.4 | 45.1 | 46.9 | 44.7 | 38.5 | 69.3 | 67.3 | 62.0 | 84.7 | 104 |
| 106 | 94.2 | 46.0 | 47.8 | 45.6 | 39.4 | 70.7 | 68.6 | 63.2 | 86.3 | 106 |
| 108 | 95.9 | 46.9 | 48.7 | 46.6 | 40.3 | 72.1 | 70.0 | 64.4 | 88.0 | 108 |
| 110 | 97.7 | 47.8 | 49.6 | 47.5 | 41.3 | 73.5 | 71.3 | 65.6 | 89.7 | 110 |
| 112 | 99.5 | 48.7 | 50.6 | 48.4 | 42.2 | 74.8 | 72.6 | 66.7 | 91.3 | 112 |
| 114 | 101.3 | 49.6 | 51.5 | 49.4 | 43.2 | 76.2 | 74.0 | 67.9 | 93.0 | 114 |
| 116 | 103.0 | 50.5 | 52.4 | 50.3 | 44.1 | 77.6 | 75.3 | 69.1 | 94.7 | 116 |
| 118 | 104.8 | 51.4 | 53.3 | 51.2 | 45.0 | 79.0 | 76.7 | 70.3 | 96.4 | 118 |
| 120 | 106.6 | 52.3 | 54.3 | 52.2 | 46.0 | 80.3 | 78.0 | 71.5 | 98.0 | 120 |
| 122 | 108.4 | 53.2 | 55.2 | 53.1 | 46.9 | 81.7 | 79.3 | 72.7 | 99.7 | 122 |
| 124 | 110.1 | 54.1 | 56.1 | 54.1 | 47.9 | 83.1 | 80.7 | 73.9 | 101.4 | 124 |
| 126 | 111.9 | 55.0 | 57.0 | 55.0 | 48.8 | 84.5 | 82.0 | 75.1 | 103.0 | 162 |
| 128 | 113.7 | 55.9 | 58.0 | 55.9 | 49.8 | 85.8 | 83.4 | 76.3 | 104.7 | 128 |
| 130 | 115.5 | 56.8 | 58.9 | 56.9 | 50.7 | 87.2 | 84.7 | 77.5 | 106.4 | 130 |
| 132 | 117.3 | 57.7 | 59.8 | 57.8 | 51.7 | 88.6 | 86.0 | 78.7 | 108.0 | 132 |
| 134 | 119.0 | 58.6 | 60.8 | 58.8 | 52.6 | 90.0 | 87.4 | 79.7 | 109.7 | 134 |
| 136 | 120.8 | 59.5 | 61.7 | 59.7 | 53.6 | 91.3 | 88.7 | 81.1 | 111.4 | 136 |
| 138 | 122.6 | 60.4 | 62.6 | 60.7 | 54.5 | 92.7 | 90.1 | 82.3 | 113.0 | 138 |
| 140 | 124.4 | 61.3 | 63.6 | 61.6 | 55.5 | 94.1 | 91.4 | 83.5 | 114.7 | 140 |
| 142 | 126.1 | 62.2 | 64.5 | 62.6 | 56.4 | 95.5 | 92.8 | 84.7 | 116.4 | 142 |
| 144 | 127.9 | 63.1 | 65.4 | 63.5 | 57.4 | 96.8 | 94.1 | 85.9 | 118.0 | 144 |
| 146 | 129.7 | 64.0 | 66.4 | 64.5 | 58.3 | 98.2 | 95.4 | 87.1 | 119.7 | 146 |
| 148 | 131.5 | 65.0 | 67.3 | 65.4 | 59.3 | 99.6 | 96.8 | 88.3 | 121.4 | 148 |
| 150 | 133.2 | 65.9 | 68.3 | 66.4 | 60.2 | 101.0 | 98.1 | 89.5 | 123.0 | 150 |
| 152 | 135.0 | 66.8 | 69.2 | 67.3 | 61.2 | 102.3 | 99.5 | 90.8 | 124.7 | 152 |
| 154 | 136.8 | 67.7 | 70.1 | 68.3 | 62.1 | 103.7 | 100.8 | 92.0 | 126.4 | 154 |
| 156 | 138.6 | 68.6 | 71.1 | 69.2 | 63.1 | 105.1 | 102.2 | 93.2 | 128.0 | 156 |
| 158 | 140.3 | 69.5 | 72.0 | 70.2 | 64.1 | 106.5 | 103.5 | 94.4 | 129.7 | 158 |
| 160 | 142.1 | 70.4 | 73.0 | 71.2 | 65.0 | 107.9 | 104.8 | 95.6 | 131.4 | 160 |
| 162 | 143.9 | 71.4 | 73.9 | 72.1 | 66.0 | 109.2 | 106.2 | 96.8 | 133.0 | 162 |
| 164 | 145.7 | 72.3 | 74.9 | 73.1 | 66.9 | 110.6 | 107.5 | 98.0 | 134.7 | 164 |
| 166 | 147.5 | 73.2 | 75.8 | 74.0 | 67.9 | 112.0 | 108.9 | 99.2 | 136.4 | 166 |
| 168 | 149.2 | 74.1 | 76.8 | 75.0 | 68.9 | 113.4 | 110.2 | 100.4 | 138.0 | 168 |

9

## *Munson and Walker's table.*—Continued.

(Expressed in milligrams.)

| CUPROUS OXIDE ($Cu_2O$) | COPPER (Cu) | DEXTROSE (*d*-GLUCOSE) | INVERT SUGAR | INVERT SUGAR AND SUCROSE | | LACTOSE | LACTOSE AND SUCROSE | | MALTOSE | CUPROUS OXIDE ($Cu_2O$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar | 2 grams total sugar | $C_{12}H_{22}O_{11}+H_2O$ | 1 lactose, 4 sucrose | 1 lactose, 12 sucrose | $C_{12}H_{22}O_{11}+H_2O$ | |
| 170 | 151.0 | 75.1 | 77.7 | 76.0 | 69.8 | 114.8 | 111.6 | 101.6 | 139.7 | 170 |
| 172 | 152.8 | 76.0 | 78.7 | 76.9 | 70.8 | 116.1 | 112.9 | 102.8 | 141.4 | 172 |
| 174 | 154.6 | 76.9 | 79.6 | 77.9 | 71.7 | 117.5 | 114.3 | 104.1 | 143.0 | 174 |
| 176 | 156.3 | 77.8 | 80.6 | 78.8 | 72.7 | 118.9 | 115.6 | 105.3 | 144.7 | 176 |
| 178 | 158.1 | 78.8 | 81.5 | 79.8 | 73.7 | 120.3 | 117.0 | 106.5 | 146.4 | 178 |
| 180 | 159.9 | 79.7 | 82.5 | 80.8 | 74.6 | 121.6 | 118.3 | 107.7 | 148.0 | 180 |
| 182 | 161.7 | 80.6 | 83.4 | 81.7 | 75.6 | 123.1 | 119.7 | 108.9 | 149.7 | 182 |
| 184 | 163.4 | 81.5 | 84.4 | 82.7 | 76.6 | 124.3 | 121.0 | 110.1 | 151.4 | 184 |
| 186 | 165.2 | 82.5 | 85.3 | 83.7 | 77.6 | 125.8 | 122.4 | 111.3 | 153.0 | 186 |
| 188 | 167.0 | 83.4 | 86.3 | 84.6 | 78.5 | 127.2 | 123.7 | 112.5 | 154.7 | 188 |
| 190 | 168.8 | 84.3 | 87.2 | 85.6 | 79.5 | 128.5 | 125.1 | 113.8 | 156.4 | 190 |
| 192 | 170.5 | 85.3 | 88.2 | 86.6 | 80.5 | 129.9 | 126.4 | 115.0 | 158.0 | 192 |
| 194 | 172.3 | 86.2 | 89.2 | 87.6 | 81.4 | 131.3 | 127.8 | 116.2 | 159.7 | 194 |
| 196 | 174.1 | 87.1 | 90.1 | 88.5 | 82.4 | 132.7 | 129.2 | 117.4 | 161.4 | 196 |
| 198 | 175.9 | 88.1 | 91.1 | 89.5 | 83.4 | 134.1 | 130.5 | 118.6 | 163.0 | 198 |
| 200 | 177.7 | 89.0 | 92.0 | 90.5 | 84.4 | 135.4 | 131.9 | 119.8 | 164.7 | 200 |
| 202 | 179.4 | 89.9 | 93.0 | 91.4 | 85.3 | 136.8 | 133.2 | 121.0 | 166.4 | 202 |
| 204 | 181.2 | 90.9 | 94.0 | 92.4 | 86.3 | 138.2 | 134.6 | 122.3 | 168.0 | 204 |
| 206 | 183.0 | 91.8 | 94.9 | 93.4 | 87.3 | 139.6 | 135.9 | 123.5 | 169.7 | 206 |
| 208 | 184.8 | 92.8 | 95.9 | 94.4 | 88.3 | 141.0 | 137.3 | 124.7 | 171.4 | 208 |
| 210 | 186.5 | 93.7 | 96.9 | 95.4 | 89.2 | 142.3 | 138.6 | 126.0 | 173.0 | 210 |
| 212 | 188.3 | 94.6 | 97.8 | 96.3 | 90.2 | 143.7 | 140.0 | 127.2 | 174.7 | 212 |
| 214 | 190.1 | 95.6 | 98.8 | 97.3 | 91.2 | 145.1 | 141.4 | 128.4 | 176.4 | 214 |
| 216 | 191.9 | 96.5 | 99.8 | 98.3 | 92.2 | 146.5 | 142.7 | 129.6 | 178.0 | 216 |
| 218 | 193.6 | 97.5 | 100.8 | 99.3 | 93.2 | 147.9 | 144.1 | 130.9 | 179.7 | 218 |
| 220 | 195.4 | 98.4 | 101.7 | 100.3 | 94.2 | 149.3 | 145.4 | 132.1 | 181.4 | 220 |
| 222 | 197.2 | 99.4 | 102.7 | 101.2 | 95.1 | 150.7 | 146.8 | 133.3 | 183.0 | 222 |
| 224 | 199.0 | 100.3 | 103.7 | 102.2 | 96.1 | 152.0 | 148.1 | 134.5 | 184.7 | 224 |
| 226 | 200.7 | 101.3 | 104.6 | 103.2 | 97.1 | 153.4 | 149.5 | 135.8 | 186.4 | 226 |
| 228 | 202.5 | 102.2 | 105.6 | 104.2 | 98.1 | 154.8 | 150.8 | 137.0 | 188.0 | 228 |
| 230 | 204.3 | 103.2 | 106.6 | 105.2 | 99.1 | 156.2 | 152.2 | 138.2 | 189.7 | 230 |
| 232 | 206.1 | 104.1 | 107.6 | 106.2 | 100.1 | 157.6 | 153.6 | 139.4 | 191.3 | 232 |
| 234 | 207.9 | 105.1 | 108.6 | 107.2 | 101.1 | 159.0 | 154.9 | 140.7 | 193.0 | 234 |
| 236 | 209.6 | 106.0 | 109.5 | 108.2 | 102.1 | 160.3 | 156.3 | 141.9 | 194.7 | 236 |
| 238 | 211.4 | 107.0 | 110.5 | 109.2 | 103.1 | 161.7 | 157.6 | 143.2 | 196.3 | 238 |
| 240 | 213.2 | 108.0 | 111.5 | 110.1 | 104.0 | 163.1 | 159.0 | 144.4 | 198.0 | 240 |
| 242 | 215.0 | 108.9 | 112.5 | 111.1 | 105.0 | 164.5 | 160.3 | 145.6 | 199.7 | 242 |
| 244 | 216.7 | 109.9 | 113.5 | 112.1 | 106.0 | 165.9 | 161.7 | 146.9 | 201.3 | 244 |
| 246 | 218.5 | 110.8 | 114.5 | 113.1 | 107.0 | 167.3 | 163.1 | 148.1 | 203.0 | 246 |
| 248 | 220.3 | 111.8 | 115.4 | 114.1 | 108.0 | 168.7 | 164.4 | 149.3 | 204.7 | 248 |

*Munson and Walker's table.*—Continued. 9

(Expressed in milligrams.)

| CUPROUS OXIDE ($Cu_2O$) | COPPER (Cu) | DEXTROSE (*d*-GLUCOSE) | INVERT SUGAR | INVERT SUGAR AND SUCROSE | | LACTOSE | LACTOSE AND SUCROSE | | MALTOSE | CUPROUS OXIDE ($Cu_2O$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar | 2 grams total sugar | $C_{12}H_{22}O_{11}+H_2O$ | 1 lactose, 4 sucrose | 1 lactose, 12 sucrose | $C_{12}H_{22}O_{11}+H_2O$ | |
| 250 | 222.1 | 112.8 | 116.4 | 115.1 | 109.0 | 170.1 | 165.8 | 150.6 | 206.3 | 250 |
| 252 | 223.8 | 113.7 | 117.4 | 116.1 | 110.0 | 171.5 | 167.2 | 151.8 | 208.0 | 252 |
| 254 | 225.6 | 114.7 | 118.4 | 117.1 | 111.0 | 172.8 | 168.5 | 153.1 | 209.7 | 254 |
| 256 | 227.4 | 115.7 | 119.4 | 118.1 | 112.0 | 174.2 | 169.9 | 154.3 | 211.3 | 256 |
| 258 | 229.2 | 116.6 | 120.4 | 119.1 | 113.0 | 175.6 | 171.3 | 155.5 | 213.0 | 258 |
| 260 | 231.0 | 117.6 | 121.4 | 120.1 | 114.0 | 177.0 | 172.6 | 156.8 | 214.7 | 260 |
| 262 | 232.7 | 118.6 | 122.4 | 121.1 | 115.0 | 178.4 | 174.0 | 158.0 | 216.3 | 262 |
| 264 | 234.5 | 119.5 | 123.4 | 122.1 | 116.0 | 179.8 | 175.3 | 159.3 | 218.0 | 264 |
| 266 | 236.3 | 120.5 | 124.4 | 123.1 | 117.0 | 181.2 | 176.7 | 160.5 | 219.7 | 266 |
| 268 | 238.1 | 121.5 | 125.4 | 124.1 | 118.0 | 182.6 | 178.1 | 161.8 | 221.3 | 268 |
| 270 | 239.8 | 122.5 | 126.4 | 125.1 | 119.0 | 184.0 | 179.4 | 163.0 | 223.0 | 270 |
| 272 | 241.6 | 123.4 | 127.4 | 126.2 | 120.0 | 185.3 | 180.8 | 164.3 | 224.6 | 272 |
| 274 | 243.4 | 124.4 | 128.4 | 127.2 | 121.1 | 186.7 | 182.2 | 165.5 | 226.3 | 274 |
| 276 | 245.2 | 125.4 | 129.4 | 128.2 | 122.1 | 188.1 | 183.5 | 166.8 | 228.0 | 276 |
| 278 | 246.9 | 126.4 | 130.4 | 129.2 | 123.1 | 189.5 | 184.9 | 168.0 | 229.6 | 278 |
| 280 | 248.7 | 127.3 | 131.4 | 130.2 | 124.1 | 190.9 | 186.3 | 169.3 | 231.3 | 280 |
| 282 | 250.5 | 128.3 | 132.4 | 131.2 | 125.1 | 192.3 | 187.6 | 170.5 | 233.0 | 282 |
| 284 | 252.3 | 129.3 | 133.4 | 132.2 | 126.1 | 193.7 | 189.0 | 171.8 | 234.6 | 284 |
| 286 | 254.0 | 130.3 | 134.4 | 133.2 | 127.1 | 195.1 | 190.4 | 173.0 | 236.3 | 286 |
| 288 | 255.8 | 131.3 | 135.4 | 134.3 | 128.1 | 196.5 | 191.7 | 174.3 | 238.0 | 288 |
| 290 | 257.6 | 132.3 | 136.4 | 135.3 | 129.2 | 197.8 | 193.1 | 175.5 | 239.6 | 290 |
| 292 | 259.4 | 133.2 | 137.4 | 136.3 | 130.2 | 199.2 | 194.4 | 176.8 | 241.3 | 292 |
| 294 | 261.2 | 934.2 | 138.4 | 137.3 | 131.2 | 200.6 | 195.8 | 178.1 | 242.9 | 294 |
| 296 | 262.9 | 135.2 | 139.4 | 138.3 | 132.2 | 202.0 | 197.2 | 179.3 | 244.6 | 296 |
| 298 | 264.7 | 136.2 | 140.5 | 139.4 | 133.2 | 203.4 | 198.6 | 180.6 | 246.3 | 298 |
| 300 | 266.5 | 137.2 | 141.5 | 140.4 | 134.2 | 204.8 | 199.9 | 181.8 | 247.9 | 300 |
| 302 | 268.3 | 138.2 | 142.5 | 141.4 | 135.3 | 206.2 | 201.3 | 183.1 | 249.6 | 302 |
| 304 | 270.0 | 139.2 | 143.5 | 142.4 | 136.3 | 207.6 | 202.7 | 184.4 | 251.3 | 304 |
| 306 | 271.8 | 140.2 | 144.5 | 143.4 | 137.3 | 209.0 | 204.0 | 185.6 | 252.9 | 306 |
| 308 | 273.6 | 141.2 | 145.5 | 144.5 | 138.3 | 210.4 | 205.4 | 186.9 | 254.6 | 308 |
| 310 | 275.4 | 142.2 | 146.6 | 145.5 | 139.4 | 211.8 | 206.8 | 188.1 | 256.3 | 310 |
| 312 | 277.1 | 143.2 | 147.6 | 146.5 | 140.4 | 213.2 | 208.1 | 189.4 | 257.9 | 312 |
| 314 | 278.9 | 144.2 | 148.6 | 147.6 | 141.4 | 214.6 | 209.5 | 190.7 | 259.6 | 314 |
| 316 | 280.7 | 145.2 | 149.6 | 148.6 | 142.4 | 216.0 | 210.9 | 191.9 | 261.2 | 316 |
| 318 | 282.5 | 146.2 | 150.7 | 149.6 | 143.5 | 217.3 | 212.2 | 193.2 | 262.9 | 318 |
| 320 | 284.2 | 147.2 | 151.7 | 150.7 | 144.5 | 218.7 | 213.6 | 194.4 | 264.6 | 320 |
| 322 | 286.0 | 148.2 | 152.7 | 151.7 | 145.5 | 220.1 | 215.5 | 195.7 | 266.2 | 322 |
| 324 | 287.8 | 149.2 | 153.7 | 152.7 | 146.6 | 221.5 | 216.4 | 197.0 | 267.9 | 324 |
| 326 | 289.6 | 150.2 | 154.8 | 153.8 | 147.6 | 222.9 | 217.7 | 198.2 | 269.6 | 326 |
| 328 | 291.4 | 151.2 | 155.8 | 154.8 | 148.6 | 224.3 | 219.1 | 199.5 | 271.2 | 328 |

9

## *Munson and Walker's table.*—Continued.

(Expressed in milligrams.)

| CUPROUS OXIDE ($Cu_2O$) | COPPER (Cu) | DEXTROSE (*d*-GLUCOSE) | INVERT SUGAR | INVERT SUGAR AND SUCROSE | | LACTOSE | LACTOSE AND SUCROSE | | MALTOSE | CUPROUS OXIDE ($Cu_2O$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar | 2 grams total sugar | $C_{12}H_{22}O_{11}+H_2O$ | 1 lactose, 4 sucrose | 1 lactose, 12 sucrose | $C_{12}H_{22}O_{11}+H_2O$ | |
| 330 | 293.1 | 152.2 | 156.8 | 155.8 | 149.7 | 225.7 | 220.5 | 200.8 | 272.9 | 330 |
| 332 | 294.9 | 153.2 | 157.9 | 156.9 | 150.7 | 227.1 | 221.8 | 202.0 | 274.6 | 332 |
| 334 | 296.7 | 154.2 | 158.9 | 157.9 | 151.7 | 228.5 | 223.2 | 203.3 | 276.2 | 334 |
| 336 | 298.5 | 155.2 | 159.9 | 159.0 | 152.8 | 229.9 | 224.6 | 204.6 | 277.9 | 336 |
| 338 | 300.2 | 156.3 | 161.0 | 160.0 | 153.8 | 231.3 | 226.0 | 205.9 | 279.5 | 338 |
| 340 | 302.0 | 157.3 | 162.0 | 161.0 | 154.8 | 232.7 | 227.4 | 207.1 | 281.2 | 340 |
| 342 | 303.8 | 158.3 | 163.1 | 162.1 | 155.9 | 234.1 | 228.7 | 208.4 | 282.9 | 342 |
| 344 | 305.6 | 159.3 | 164.1 | 163.1 | 156.9 | 235.5 | 230.1 | 209.7 | 284.5 | 344 |
| 346 | 307.3 | 160.3 | 165.1 | 164.2 | 158.0 | 236.9 | 231.5 | 211.0 | 286.2 | 346 |
| 348 | 309.1 | 161.4 | 166.2 | 165.2 | 159.0 | 238.3 | 232.9 | 212.2 | 287.9 | 348 |
| 350 | 310.9 | 162.4 | 167.2 | 166.3 | 160.1 | 239.7 | 234.3 | 213.5 | 289.5 | 350 |
| 352 | 312.7 | 163.4 | 168.3 | 167.3 | 161.1 | 241.1 | 235.6 | 214.8 | 291.2 | 352 |
| 354 | 314.4 | 164.4 | 169.3 | 168.4 | 162.2 | 242.5 | 237.0 | 216.1 | 292.8 | 354 |
| 356 | 316.2 | 165.4 | 170.4 | 169.4 | 163.2 | 243.9 | 238.4 | 217.3 | 294.5 | 356 |
| 358 | 318.0 | 166.5 | 171.4 | 170.5 | 164.3 | 245.3 | 239.8 | 218.6 | 296.2 | 358 |
| 360 | 319.8 | 167.5 | 172.5 | 171.5 | 165.3 | 246.7 | 241.2 | 219.9 | 297.8 | 360 |
| 362 | 321.6 | 168.5 | 173.5 | 172.6 | 166.4 | 248.1 | 242.5 | 221.2 | 299.5 | 362 |
| 364 | 323.3 | 169.6 | 174.6 | 173.7 | 167.4 | 249.5 | 243.9 | 222.5 | 301.2 | 364 |
| 366 | 325.1 | 170.6 | 175.6 | 174.7 | 168.5 | 250.9 | 245.3 | 223.7 | 302.8 | 366 |
| 368 | 326.9 | 171.6 | 176.7 | 175.8 | 169.5 | 252.3 | 246.7 | 225.0 | 304.5 | 368 |
| 370 | 328.7 | 172.7 | 177.7 | 176.8 | 170.6 | 253.7 | 248.1 | 226.3 | 306.1 | 370 |
| 372 | 330.4 | 173.7 | 178.8 | 177.9 | 171.6 | 255.1 | 249.5 | 227.6 | 307.8 | 372 |
| 374 | 332.2 | 174.7 | 179.8 | 179.0 | 172.7 | 256.5 | 250.9 | 228.9 | 309.5 | 374 |
| 376 | 334.0 | 175.8 | 180.9 | 180.0 | 173.7 | 257.9 | 252.2 | 230.2 | 311.1 | 376 |
| 378 | 335.8 | 176.8 | 182.0 | 181.1 | 174.8 | 259.3 | 253.6 | 231.5 | 312.8 | 378 |
| 380 | 337.5 | 177.9 | 183.0 | 182.1 | 175.9 | 260.7 | 255.0 | 232.8 | 314.5 | 380 |
| 382 | 339.3 | 178.9 | 184.1 | 183.2 | 176.9 | 262.1 | 256.4 | 234.1 | 316.1 | 382 |
| 384 | 341.1 | 180.0 | 185.2 | 184.3 | 178.0 | 263.5 | 257.8 | 235.4 | 317.8 | 384 |
| 386 | 342.9 | 181.0 | 186.2 | 185.4 | 179.1 | 264.9 | 259.2 | 236.6 | 329.4 | 386 |
| 388 | 344.6 | 182.0 | 187.3 | 186.4 | 180.1 | 266.5 | 260.5 | 237.9 | 321.1 | 388 |
| 390 | 346.4 | 183.1 | 188.4 | 187.5 | 181.2 | 267.7 | 261.9 | 239.2 | 322.8 | 390 |
| 392 | 348.2 | 184.1 | 189.4 | 188.6 | 182.3 | 269.1 | 263.3 | 240.5 | 324.4 | 392 |
| 394 | 350.0 | 185.2 | 190.5 | 189.7 | 183.3 | 270.5 | 264.7 | 241.8 | 326.1 | 394 |
| 396 | 351.8 | 186.2 | 191.6 | 190.7 | 184.4 | 271.9 | 266.1 | 243.1 | 327.7 | 396 |
| 398 | 353.5 | 187.3 | 192.7 | 191.8 | 185.5 | 273.3 | 267.5 | 244.4 | 329.4 | 398 |
| 400 | 355.3 | 188.4 | 193.7 | 192.9 | 186.5 | 274.7 | 268.9 | 245.7 | 331.1 | 400 |
| 402 | 357.1 | 189.4 | 194.8 | 194.0 | 187.6 | 276.1 | 270.3 | 247.0 | 332.7 | 402 |
| 404 | 358.9 | 190.5 | 195.9 | 195.0 | 188.7 | 277.5 | 271.7 | 248.3 | 334.4 | 404 |
| 406 | 360.6 | 191.5 | 197.0 | 196.1 | 189.8 | 278.9 | 273.0 | 249.6 | 336.0 | 406 |
| 408 | 362.4 | 192.6 | 198.1 | 197.2 | 190.8 | 280.3 | 274.4 | 251.0 | 337.7 | 408 |

## *Munson and Walker's table.*—Continued.

(Expressed in milligrams.)

| CUPROUS OXIDE ($Cu_2O$) | COPPER (Cu) | DEXTROSE (*d*-GLUCOSE) | INVERT SUGAR | INVERT SUGAR AND SUCROSE |  | LACTOSE | LACTOSE AND SUCROSE |  | MALTOSE | CUPROUS OXIDE ($Cu_2O$) |
|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | 0.4 gram total sugar | 2 grams total sugar | $C_{12}H_{22}O_{11}+H_2O$ | 1 lactose, 4 sucrose | 1 lactose, 12 sucrose | $C_{12}H_{22}O_{11}+H_2O$ |  |
| 410 | 364.2 | 193.7 | 199.1 | 198.3 | 191.9 | 281.7 | 275.8 | 252.3 | 339.4 | 410 |
| 412 | 366.0 | 194.7 | 200.2 | 199.4 | 193.0 | 283.2 | 277.2 | 253.6 | 341.0 | 412 |
| 414 | 367.7 | 195.8 | 201.3 | 200.5 | 194.1 | 284.6 | 278.6 | 254.9 | 342.7 | 414 |
| 416 | 369.5 | 196.8 | 202.4 | 201.6 | 105.2 | 286.0 | 280.0 | 256.2 | 344.4 | 416 |
| 418 | 371.3 | 197.9 | 203.5 | 202.6 | 196.2 | 287.4 | 281.4 | 257.5 | 346.0 | 418 |
| 420 | 373.1 | 199.0 | 204.6 | 203.7 | 197.3 | 288.8 | 282.8 | 258.8 | 347.7 | 420 |
| 422 | 374.8 | 200.1 | 205.7 | 204.8 | 198.4 | 290.2 | 284.2 | 260.1 | 349.3 | 422 |
| 424 | 376.6 | 201.1 | 206.7 | 205.9 | 199.5 | 291.6 | 285.6 | 261.4 | 351.0 | 424 |
| 426 | 378.4 | 202.2 | 207.8 | 207.0 | 200.6 | 293.0 | 287.0 | 262.7 | 352.7 | 426 |
| 428 | 380.2 | 203.3 | 208.9 | 208.1 | 201.7 | 294.4 | 288.4 | 264.0 | 354.3 | 428 |
| 430 | 382.0 | 204.4 | 310.0 | 209.2 | 202.7 | 295.8 | 289.8 | 265.4 | 356.0 | 430 |
| 432 | 383.7 | 205.5 | 211.1 | 210.3 | 203.8 | 297.2 | 291.2 | 266.6 | 357.6 | 432 |
| 434 | 385.5 | 206.5 | 212.2 | 211.4 | 204.9 | 298.6 | 292.6 | 268.0 | 359.3 | 434 |
| 436 | 387.3 | 207.6 | 213.3 | 212.5 | 206.0 | 300.0 | 294.0 | 269.3 | 361.0 | 436 |
| 438 | 389.1 | 208.7 | 214.4 | 213.6 | 207.1 | 301.4 | 295.4 | 270.6 | 362.6 | 438 |
| 440 | 390.8 | 209.8 | 215.5 | 214.7 | 208.2 | 302.8 | 296.8 | 272.0 | 364.3 | 440 |
| 442 | 392.6 | 210.9 | 216.6 | 215.8 | 209.3 | 304.2 | 298.2 | 273.3 | 365.9 | 442 |
| 444 | 394.4 | 212.0 | 217.8 | 216.9 | 210.4 | 305.6 | 299.6 | 274.6 | 367.6 | 444 |
| 446 | 396.2 | 213.1 | 218.9 | 218.0 | 211.5 | 307.0 | 301.0 | 275.9 | 369.3 | 446 |
| 448 | 397.9 | 214.1 | 220.0 | 219.1 | 212.6 | 308.4 | 302.4 | 277.2 | 370.9 | 448 |
| 450 | 399.7 | 215.2 | 221.1 | 220.2 | 213.7 | 309.9 | 303.8 | 278.6 | 372.6 | 450 |
| 452 | 401.5 | 216.3 | 222.2 | 221.4 | 214.8 | 311.3 | 305.2 | 279.9 | 374.2 | 452 |
| 454 | 403.3 | 217.4 | 223.3 | 222.5 | 215.9 | 312.7 | 306.6 | 281.2 | 375.9 | 454 |
| 456 | 405.1 | 218.5 | 224.4 | 223.6 | 217.0 | 314.1 | 308.0 | 282.5 | 377.6 | 456 |
| 458 | 406.8 | 219.6 | 225.5 | 224.7 | 218.1 | 315.5 | 309.4 | 283.9 | 379.2 | 458 |
| 460 | 408.6 | 220.7 | 226.7 | 225.8 | 219.2 | 316.9 | 310.8 | 285.2 | 380.9 | 460 |
| 462 | 410.4 | 221.8 | 227.8 | 226.9 | 220.3 | 318.3 | 312.2 | 286.5 | 382.5 | 462 |
| 464 | 412.2 | 222.9 | 228.9 | 228.1 | 221.4 | 319.7 | 313.6 | 287.8 | 384.2 | 464 |
| 466 | 413.9 | 224.0 | 230.0 | 229.2 | 222.5 | 321.1 | 315.0 | 289.2 | 385.9 | 466 |
| 468 | 415.7 | 225.1 | 231.2 | 230.3 | 223.7 | 322.5 | 316.4 | 290.5 | 387.5 | 468 |
| 470 | 417.5 | 226.2 | 232.3 | 231.4 | 224.8 | 323.9 | 317.7 | 291.8 | 389.2 | 470 |
| 472 | 419.3 | 227.4 | 233.4 | 232.5 | 225.9 | 325.3 | 319.1 | 293.2 | 390.8 | 472 |
| 474 | 421.0 | 228.3 | 234.5 | 233.7 | 227.0 | 326.8 | 320.5 | 294.5 | 392.5 | 474 |
| 476 | 422.8 | 229.6 | 235.7 | 234.8 | 228.1 | 328.2 | 321.9 | 295.8 | 394.2 | 476 |
| 478 | 424.6 | 230.7 | 236.8 | 235.9 | 229.2 | 329.6 | 323.3 | 297.1 | 395.8 | 478 |
| 480 | 426.4 | 231.8 | 237.9 | 237.1 | 230.3 | 331.0 | 324.7 | 298.5 | 397.5 | 480 |
| 482 | 428.1 | 232.9 | 239.1 | 238.2 | 231.5 | 332.4 | 326.1 | 299.8 | 399.1 | 482 |
| 484 | 429.9 | 234.1 | 240.2 | 239.3 | 232.6 | 333.8 | 327.5 | 301.1 | 400.8 | 484 |
| 486 | 431.7 | 235.2 | 241.4 | 240.5 | 233.7 | 335.2 | 328.9 | 302.5 | 402.4 | 486 |
| 488 | 433.5 | 236.3 | 242.5 | 241.6 | 234.8 | 336.6 | 330.3 | 303.8 | 404.1 | 488 |
| 490 | 435.3 | 237.4 | 243.6 | 242.7 | 236.0 | 338.0 | 331.7 | 305.1 | 405.8 | 490 |

## 10 *Herzfeld's table for determining invert sugar in raw sugars*[1] *(invert sugar not to exceed 1.5 per cent).*

| COPPER (Cu) | INVERT SUGAR | COPPER (Cu) | INVERT SUGAR | COPPER (Cu) | INVERT SUGAR | COPPER (Cu) | INVERT SUGAR | COPPER (Cu) | INVERT SUGAR |
|---|---|---|---|---|---|---|---|---|---|
| *mg.* | *per cent* | *mg.* | *per cent* | *mg.* | *per cent* | *mg.* | *per cent* | *mg.* | *per cent* |
| 50 | 0.050 | 110 | 0.351 | 170 | 0.680 | 230 | 0.013 | 290 | 1.357 |
| 52 | 0.058 | 112 | 0.361 | 172 | 0.692 | 232 | 1.024 | 292 | 1.368 |
| 54 | 0.066 | 114 | 0.371 | 174 | 0.704 | 234 | 1.036 | 294 | 1.380 |
| 56 | 0.074 | 116 | 0.381 | 176 | 0.715 | 236 | 1.047 | 296 | 1.391 |
| 58 | 0.082 | 118 | 0.392 | 178 | 0.726 | 238 | 1.058 | 298 | 1.403 |
| 60 | 0.090 | 120 | 0.402 | 180 | 0.737 | 240 | 1.070 | 300 | 1.414 |
| 62 | 0.098 | 122 | 0.412 | 182 | 0.748 | 242 | 1.081 | 302 | 1.425 |
| 64 | 0.108 | 124 | 0.423 | 184 | 0.759 | 244 | 1.093 | 304 | 1.437 |
| 66 | 0.118 | 126 | 0.433 | 186 | 0.770 | 246 | 1.104 | 306 | 1.448 |
| 68 | 0.128 | 128 | 0.443 | 188 | 0.781 | 248 | 1.116 | 308 | 1.460 |
| 70 | 0.138 | 130 | 0.453 | 190 | 0.792 | 250 | 1.127 | 310 | 1.471 |
| 72 | 0.148 | 132 | 0.463 | 192 | 0.803 | 252 | 1.139 | 312 | 1.483 |
| 74 | 0.157 | 134 | 0.473 | 194 | 0.814 | 254 | 1.150 | 314 | 1.494 |
| 76 | 0.167 | 136 | 0.483 | 196 | 0.825 | 256 | 1.162 | | |
| 78 | 0.177 | 138 | 0.493 | 198 | 0.836 | 258 | 1.173 | | |
| 80 | 0.187 | 140 | 0.503 | 200 | 0.847 | 260 | 1.185 | | |
| 82 | 0.197 | 142 | 0.515 | 202 | 0.858 | 262 | 1.196 | | |
| 84 | 0.208 | 144 | 0.527 | 204 | 0.869 | 264 | 1.207 | | |
| 86 | 0.219 | 146 | 0.538 | 206 | 0.880 | 266 | 1.219 | | |
| 88 | 0.231 | 148 | 0.550 | 208 | 0.891 | 268 | 1.231 | | |
| 90 | 0.242 | 150 | 0.562 | 210 | 0.902 | 270 | 1.242 | | |
| 92 | 0.254 | 152 | 0.574 | 212 | 0.913 | 272 | 1.253 | | |
| 94 | 0.265 | 154 | 0.586 | 214 | 0.924 | 274 | 1.265 | | |
| 96 | 0.277 | 156 | 0.598 | 216 | 0.935 | 276 | 1.276 | | |
| 98 | 0.288 | 158 | 0.609 | 218 | 0.946 | 278 | 1.288 | | |
| 100 | 0.300 | 160 | 0.621 | 220 | 0.957 | 280 | 1.299 | | |
| 102 | 0.310 | 162 | 0.633 | 222 | 0.968 | 282 | 1.311 | | |
| 104 | 0.320 | 164 | 0.645 | 224 | 0.979 | 284 | 1.322 | | |
| 106 | 0.330 | 166 | 0.657 | 226 | 0.990 | 286 | 1.334 | | |
| 108 | 0.340 | 168 | 0.669 | 228 | 1.001 | 288 | 1.345 | | |

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[1] Z. Ver. Ruebenzucker-Ind., **35** (N.F. 22); 1012 (1885).

*Meissl and Hiller's*[1] *factors for determining invert sugar in materials in which, of the total sugars present, more than 1.5 per cent is invert sugar, and less than 98.5 per cent is sucrose.* 11

| RATIO OF SUCROSE TO INVERT SUGAR=R:I. | APPROXIMATE ABSOLUTE WEIGHT OF INVERT SUGAR (Z) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 200 mg | 175 mg | 150 mg | 125 mg | 100 mg | 75 mg | 50 mg |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 0:100 | 56.4 | 55.4 | 54.5 | 53.8 | 53.2 | 53.0 | 53.0 |
| 10:90 | 56.3 | 55.3 | 54.4 | 53.8 | 53.2 | 52.9 | 52.9 |
| 20:80 | 56.2 | 55.2 | 54.3 | 53.7 | 53.2 | 52.7 | 52.7 |
| 30:70 | 56.1 | 55.1 | 54.2 | 53.7 | 53.2 | 52.6 | 52.6 |
| 40:60 | 55.9 | 55.0 | 54.1 | 53.6 | 53.1 | 52.5 | 52.4 |
| 50:50 | 55.7 | 54.9 | 54.0 | 53.5 | 53.1 | 52.3 | 52.2 |
| 60:40 | 55.6 | 54.7 | 53.8 | 53.2 | 52.8 | 52.1 | 51.9 |
| 70:30 | 55.5 | 54.5 | 53.5 | 52.9 | 52.5 | 51.9 | 51.6 |
| 80:20 | 55.4 | 54.3 | 53.3 | 52.7 | 52.2 | 51.7 | 51.3 |
| 90:10 | 54.6 | 53.6 | 53.1 | 52.6 | 52.1 | 51.6 | 51.2 |
| 91:9 | 54.1 | 53.6 | 52.6 | 52.1 | 51.6 | 51.2 | 50.7 |
| 92:8 | 53.6 | 53.1 | 52.1 | 51.6 | 51.2 | 50.7 | 50.3 |
| 93:7 | 53.6 | 53.1 | 52.1 | 51.2 | 50.7 | 50.3 | 49.8 |
| 94:6 | 53.1 | 52.6 | 51.6 | 50.7 | 50.3 | 49.8 | 48.9 |
| 95:5 | 52.6 | 52.1 | 51.2 | 50.3 | 49.4 | 48.9 | 48.5 |
| 96:4 | 52.1 | 51.2 | 50.7 | 49.8 | 48.9 | 47.7 | 46.9 |
| 97:3 | 50.7 | 50.3 | 49.8 | 48.9 | 47.7 | 46.2 | 45.1 |
| 98:2 | 49.9 | 48.9 | 48.5 | 47.3 | 45.8 | 43.3 | 40.0 |
| 99:1 | 47.7 | 47.3 | 46.5 | 45.1 | 43.3 | 41.2 | 38.1 |

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[1] Z. Ver. Ruebenzucker-Ind., **39** (N.F. 26), 734, (1889).

**12**

### *Wein's table*[1] *for the determination of maltose.*

(Expressed in milligrams.)

| COPPER | CUPROUS OXIDE | MALTOSE | COPPER | CUPROUS OXIDE | MALTOSE | COPPER | CUPROUS OXIDE | MALTOSE |
|---|---|---|---|---|---|---|---|---|
| 32 | 36.0 | 27.0 | 122 | 137.4 | 106.2 | 212 | 238.7 | 186.8 |
| 34 | 38.3 | 28.7 | 124 | 139.6 | 108.0 | 214 | 240.9 | 188.6 |
| 36 | 40.5 | 30.5 | 126 | 141.9 | 109.8 | 216 | 243.2 | 190.4 |
| 38 | 42.8 | 32.2 | 128 | 144.1 | 111.6 | 218 | 245.4 | 192.1 |
| 40 | 45.0 | 33.9 | 130 | 146.4 | 113.4 | 220 | 247.7 | 193.9 |
| 42 | 47.3 | 35.7 | 132 | 148.6 | 115.2 | 222 | 249.9 | 195.7 |
| 44 | 49.5 | 37.4 | 134 | 150.9 | 117.0 | 224 | 252.4 | 197.5 |
| 46 | 51.8 | 39.1 | 136 | 153.1 | 118.8 | 226 | 254.4 | 199.3 |
| 48 | 54.0 | 40.9 | 138 | 155.4 | 120.6 | 228 | 256.7 | 201.1 |
| 50 | 56.3 | 42.6 | 140 | 157.6 | 122.4 | 230 | 258.9 | 202.9 |
| 52 | 58.5 | 44.4 | 142 | 159.9 | 124.2 | 232 | 261.2 | 204.7 |
| 54 | 60.8 | 46.1 | 144 | 162.1 | 126.0 | 234 | 263.4 | 206.5 |
| 56 | 63.0 | 47.8 | 146 | 164.4 | 127.8 | 236 | 265.7 | 208.3 |
| 58 | 65.3 | 49.6 | 148 | 166.6 | 129.6 | 238 | 268.0 | 210.0 |
| 60 | 67.6 | 51.3 | 150 | 168.9 | 131.4 | 240 | 270.2 | 211.8 |
| 62 | 69.8 | 53.1 | 152 | 171.1 | 133.2 | 242 | 272.5 | 213.6 |
| 64 | 72.1 | 54.8 | 154 | 173.4 | 135.0 | 244 | 274.7 | 215.4 |
| 66 | 74.3 | 56.6 | 156 | 175.6 | 136.8 | 246 | 277.0 | 217.2 |
| 68 | 76.6 | 58.3 | 158 | 177.9 | 138.6 | 248 | 279.2 | 219.0 |
| 70 | 78.8 | 60.1 | 160 | 180.1 | 140.4 | 250 | 281.5 | 220.8 |
| 72 | 81.1 | 61.8 | 162 | 182.4 | 142.2 | 252 | 283.7 | 222.6 |
| 74 | 83.3 | 63.6 | 164 | 184.6 | 144.0 | 254 | 286.0 | 224.4 |
| 76 | 85.6 | 65.4 | 166 | 186.9 | 145.8 | 256 | 288.2 | 226.2 |
| 78 | 87.8 | 67.1 | 168 | 189.1 | 147.8 | 258 | 290.5 | 228.0 |
| 80 | 90.1 | 68.9 | 170 | 191.4 | 149.4 | 260 | 292.7 | 229.8 |
| 82 | 92.3 | 70.6 | 172 | 193.6 | 151.2 | 262 | 295.0 | 231.6 |
| 84 | 94.6 | 72.4 | 174 | 195.9 | 152.9 | 264 | 297.2 | 233.4 |
| 86 | 96.8 | 74.1 | 176 | 198.1 | 154.7 | 266 | 299.5 | 235.2 |
| 88 | 99.1 | 75.9 | 178 | 200.4 | 156.5 | 268 | 301.7 | 237.0 |
| 90 | 101.3 | 77.7 | 180 | 202.6 | 158.3 | 270 | 304.0 | 238.8 |
| 92 | 103.6 | 79.5 | 182 | 204.9 | 160.1 | 272 | 306.2 | 240.6 |
| 94 | 105.8 | 81.2 | 184 | 207.1 | 161.8 | 274 | 308.5 | 242.4 |
| 96 | 108.1 | 83.0 | 186 | 209.4 | 163.6 | 276 | 310.7 | 244.2 |
| 98 | 110.3 | 84.8 | 188 | 211.7 | 165.4 | 278 | 313.0 | 246.0 |
| 100 | 112.6 | 86.6 | 190 | 213.9 | 167.2 | 280 | 315.2 | 247.8 |
| 102 | 114.8 | 88.4 | 192 | 216.2 | 169.0 | 282 | 317.5 | 249.6 |
| 104 | 117.1 | 90.1 | 194 | 218.4 | 170.7 | 284 | 319.7 | 251.3 |
| 106 | 119.3 | 91.9 | 196 | 220.7 | 172.5 | 286 | 322.0 | 253.1 |
| 108 | 121.6 | 93.7 | 198 | 222.9 | 174.3 | 288 | 324.2 | 254.9 |
| 110 | 123.8 | 95.5 | 200 | 225.2 | 176.1 | 290 | 326.5 | 256.6 |
| 112 | 126.1 | 97.3 | 202 | 227.4 | 177.9 | 292 | 328.7 | 258.4 |
| 114 | 128.3 | 99.0 | 204 | 229.7 | 179.6 | 294 | 331.0 | 260.2 |
| 116 | 130.6 | 100.8 | 206 | 231.9 | 181.4 | 296 | 333.2 | 262.0 |
| 118 | 132.8 | 102.6 | 208 | 234.2 | 183.2 | 298 | 335.5 | 263.7 |
| 120 | 135.1 | 104.4 | 210 | 236.4 | 185.0 | 300 | 337.8 | 265.5 |

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[1] Tables for the Quantitative Estimation of the Sugars. Translated by Frew, 1896, p. 26.

## *Soxhlet and Wein's table*[1] *for the determination of lactose.* 13

(Expressed in milligrams.)

| COPPER | LACTOSE | COPPER | LACTOSE | COPPER | LACTOSE | COPPER | LACTOSE | COPPER | LACTOSE |
|---|---|---|---|---|---|---|---|---|---|
| 100 | 71.6 | 160 | 116.4 | 220 | 161.9 | 280 | 208.3 | 340 | 255.7 |
| 102 | 73.1 | 162 | 117.9 | 222 | 163.4 | 282 | 209.9 | 342 | 257.4 |
| 104 | 74.6 | 164 | 119.4 | 224 | 164.9 | 284 | 211.5 | 344 | 259.0 |
| 106 | 76.1 | 166 | 120.9 | 226 | 166.4 | 286 | 213.1 | 346 | 260.6 |
| 108 | 77.6 | 168 | 122.4 | 228 | 167.9 | 288 | 214.7 | 348 | 262.3 |
| 110 | 79.0 | 170 | 123.9 | 230 | 169.4 | 290 | 216.3 | 350 | 263.9 |
| 112 | 80.5 | 172 | 125.5 | 232 | 170.9 | 292 | 217.9 | 352 | 265.5 |
| 114 | 82.0 | 174 | 127.0 | 234 | 172.4 | 294 | 219.5 | 354 | 267.2 |
| 116 | 83.5 | 176 | 128.5 | 236 | 173.9 | 296 | 221.1 | 356 | 268.8 |
| 118 | 85.0 | 178 | 130.1 | 238 | 175.4 | 298 | 222.7 | 358 | 270.4 |
| 120 | 86.4 | 180 | 131.6 | 240 | 176.9 | 300 | 224.4 | 360 | 272.1 |
| 122 | 87.9 | 182 | 133.1 | 242 | 178.5 | 302 | 225.9 | 362 | 273.7 |
| 124 | 89.4 | 184 | 134.7 | 244 | 180.1 | 304 | 227.5 | 364 | 275.3 |
| 126 | 90.9 | 186 | 136.2 | 246 | 181.6 | 306 | 229.1 | 366 | 277.1 |
| 128 | 92.4 | 188 | 137.7 | 248 | 183.2 | 308 | 230.6 | 368 | 278.8 |
| 130 | 93.8 | 190 | 139.3 | 250 | 184.8 | 310 | 232.2 | 370 | 280.5 |
| 132 | 95.3 | 192 | 140.8 | 252 | 186.3 | 312 | 233.7 | 372 | 282.2 |
| 134 | 96.9 | 194 | 142.3 | 254 | 187.9 | 314 | 235.3 | 374 | 283.9 |
| 136 | 98.3 | 196 | 143.9 | 256 | 189.4 | 316 | 236.8 | 376 | 285.7 |
| 138 | 99.8 | 198 | 145.4 | 258 | 191.0 | 318 | 238.4 | 378 | 287.4 |
| 140 | 101.3 | 200 | 146.9 | 260 | 192.5 | 320 | 240.0 | 380 | 289.1 |
| 142 | 102.8 | 202 | 148.5 | 262 | 194.1 | 322 | 241.5 | 382 | 290.8 |
| 144 | 104.3 | 204 | 150.0 | 264 | 195.7 | 324 | 243.1 | 384 | 292.5 |
| 146 | 105.8 | 206 | 151.5 | 266 | 197.2 | 326 | 244.6 | 386 | 294.2 |
| 148 | 107.3 | 208 | 153.0 | 268 | 198.8 | 328 | 246.2 | 388 | 296.0 |
| 150 | 108.8 | 210 | 154.5 | 270 | 200.3 | 330 | 247.7 | 390 | 297.7 |
| 152 | 110.3 | 212 | 156.0 | 272 | 201.9 | 332 | 249.2 | 392 | 299.4 |
| 154 | 111.9 | 214 | 157.5 | 274 | 203.5 | 334 | 250.8 | 394 | 301.1 |
| 156 | 113.4 | 216 | 159.0 | 276 | 205.1 | 336 | 252.5 | 396 | 302.8 |
| 158 | 114.9 | 218 | 160.4 | 278 | 206.7 | 338 | 254.1 | 398 | 304.6 |
|  |  |  |  |  |  |  |  | 400 | 306.3 |

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[1] Wein, Tables for the Quantitative Estimation of the Sugars. Translated by Frew, 1896, p. 33.

14

*Allihn's table*[1] *for the determination of dextrose.*

(Expressed in milligrams.)

| COPPER | CUPROUS OXIDE | DEXTROSE | COPPER | CUPROUS OXIDE | DEXTROSE | COPPER | CUPROUS OXIDE | DEXTROSE |
|---|---|---|---|---|---|---|---|---|
| 12 | 13.5 | 7.1 | 102 | 114.8 | 51.9 | 192 | 216.2 | 98.4 |
| 14 | 15.8 | 8.1 | 104 | 117.1 | 52.9 | 194 | 218.4 | 99.4 |
| 16 | 18.0 | 9.0 | 106 | 119.3 | 54.0 | 196 | 220.7 | 100.5 |
| 18 | 20.3 | 10.0 | 108 | 121.6 | 55.0 | 198 | 222.9 | 101.5 |
| 20 | 22.5 | 11.0 | 110 | 123.8 | 56.0 | 200 | 225.2 | 102.6 |
| 22 | 24.8 | 12.0 | 112 | 126.1 | 57.0 | 202 | 227.4 | 103.7 |
| 24 | 27.0 | 13.0 | 114 | 128.3 | 58.0 | 204 | 229.7 | 104.7 |
| 26 | 29.3 | 14.0 | 116 | 130.6 | 59.1 | 206 | 231.9 | 105.8 |
| 28 | 31.5 | 15.0 | 118 | 132.8 | 60.1 | 208 | 234.2 | 106.8 |
| 30 | 33.8 | 16.0 | 120 | 135.1 | 61.1 | 210 | 236.4 | 107.9 |
| 32 | 36.0 | 17.0 | 122 | 137.4 | 62.1 | 212 | 238.7 | 109.0 |
| 34 | 38.3 | 18.0 | 124 | 139.6 | 63.1 | 214 | 240.9 | 110.0 |
| 36 | 40.5 | 18.9 | 126 | 141.9 | 64.2 | 216 | 243.2 | 111.1 |
| 38 | 42.8 | 19.9 | 128 | 144.1 | 65.2 | 218 | 245.4 | 112.1 |
| 40 | 45.0 | 20.9 | 130 | 146.4 | 66.2 | 220 | 247.7 | 113.2 |
| 42 | 47.3 | 21.9 | 132 | 148.6 | 67.2 | 222 | 249.9 | 114.3 |
| 44 | 49.5 | 22.9 | 134 | 150.9 | 68.2 | 224 | 252.4 | 115.3 |
| 46 | 51.8 | 23.9 | 136 | 153.1 | 69.3 | 226 | 254.4 | 116.4 |
| 48 | 54.0 | 24.9 | 138 | 155.4 | 70.3 | 228 | 256.7 | 117.4 |
| 50 | 56.3 | 25.9 | 140 | 157.6 | 71.3 | 230 | 258.9 | 118.5 |
| 52 | 58.5 | 26.9 | 142 | 159.9 | 72.3 | 232 | 261.2 | 119.6 |
| 54 | 60.8 | 27.9 | 144 | 162.1 | 73.4 | 234 | 263.4 | 120.7 |
| 56 | 63.0 | 28.8 | 146 | 164.4 | 74.4 | 236 | 265.7 | 121.7 |
| 58 | 65.3 | 29.8 | 148 | 166.6 | 75.5 | 238 | 268.0 | 122.8 |
| 60 | 67.6 | 30.8 | 150 | 168.9 | 76.5 | 240 | 270.2 | 123.9 |
| 62 | 69.8 | 31.8 | 152 | 171.1 | 77.5 | 242 | 272.5 | 125.0 |
| 64 | 72.1 | 32.8 | 154 | 173.4 | 78.6 | 244 | 274.7 | 126.0 |
| 66 | 74.3 | 33.8 | 156 | 175.6 | 79.6 | 246 | 277.0 | 127.1 |
| 68 | 76.6 | 34.8 | 158 | 177.9 | 80.7 | 248 | 279.2 | 128.1 |
| 70 | 78.8 | 35.8 | 160 | 180.1 | 81.7 | 250 | 281.5 | 129.2 |
| 72 | 81.1 | 36.8 | 162 | 182.4 | 82.6 | 252 | 283.7 | 130.3 |
| 74 | 83.3 | 37.8 | 164 | 184.6 | 83.7 | 254 | 286.0 | 131.4 |
| 76 | 85.6 | 38.8 | 166 | 186.9 | 84.8 | 256 | 288.2 | 132.4 |
| 78 | 87.8 | 39.8 | 168 | 189.1 | 85.9 | 258 | 290.5 | 133.5 |
| 80 | 90.1 | 40.8 | 170 | 191.4 | 86.9 | 260 | 292.7 | 134.6 |
| 82 | 92.3 | 41.8 | 172 | 193.6 | 87.9 | 262 | 295.0 | 135.7 |
| 84 | 94.6 | 42.8 | 174 | 195.9 | 89.0 | 264 | 297.2 | 136.8 |
| 86 | 96.8 | 43.9 | 176 | 198.1 | 90.0 | 266 | 299.5 | 137.8 |
| 88 | 99.1 | 44.9 | 178 | 200.4 | 91.1 | 268 | 301.7 | 138.9 |
| 90 | 101.3 | 45.9 | 180 | 202.6 | 92.1 | 270 | 304.0 | 140.0 |
| 92 | 103.6 | 46.9 | 182 | 204.9 | 93.1 | 272 | 306.2 | 141.1 |
| 94 | 105.8 | 47.9 | 184 | 207.1 | 94.2 | 274 | 308.5 | 142.2 |
| 96 | 108.1 | 48.9 | 186 | 209.4 | 95.2 | 276 | 310.7 | 143.3 |
| 98 | 110.3 | 49.9 | 188 | 211.7 | 96.3 | 278 | 313.0 | 144.4 |
| 100 | 112.6 | 50.9 | 190 | 213.9 | 97.3 | 280 | 315.2 | 145.5 |

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[1] Z. Ver. Ruebenzucker-Ind., 32 (N. F. 19), 606, 865 (1882).

*Allihn's table*—Continued.

(Expressed in milligrams.)

| COPPER | CUPROUS OXIDE | DEXTROSE | COPPER | CUPROUS OXIDE | DEXTROSE | COPPER | CUPROUS OXIDE | DEXTROSE |
|---|---|---|---|---|---|---|---|---|
| 282 | 317.5 | 146.6 | 342 | 385.0 | 179.8 | 402 | 452.6 | 214.1 |
| 284 | 319.7 | 147.7 | 344 | 387.3 | 180.9 | 404 | 454.8 | 215.2 |
| 286 | 322.0 | 148.8 | 346 | 389.6 | 182.1 | 406 | 457.1 | 216.4 |
| 288 | 324.2 | 149.9 | 348 | 391.8 | 183.2 | 408 | 459.4 | 217.5 |
| 290 | 326.5 | 151.0 | 350 | 394.0 | 184.3 | 410 | 461.6 | 218.7 |
| 292 | 328.7 | 152.1 | 352 | 396.3 | 185.4 | 412 | 463.8 | 219.9 |
| 294 | 331.0 | 153.2 | 354 | 398.6 | 186.6 | 414 | 466.1 | 221.0 |
| 296 | 333.3 | 154.3 | 356 | 400.8 | 187.7 | 416 | 468.4 | 222.2 |
| 298 | 335.5 | 155.4 | 358 | 403.1 | 188.9 | 418 | 470.6 | 223.3 |
| 300 | 337.8 | 156.5 | 360 | 405.3 | 190.0 | 420 | 472.9 | 224.5 |
| 302 | 340.0 | 157.6 | 362 | 407.6 | 191.1 | 422 | 475.6 | 225.7 |
| 304 | 342.3 | 158.7 | 364 | 409.8 | 192.3 | 424 | 477.4 | 226.9 |
| 306 | 344.5 | 159.8 | 366 | 412.1 | 193.4 | 426 | 479.6 | 228.0 |
| 308 | 346.8 | 160.9 | 368 | 414.3 | 194.6 | 428 | 481.9 | 229.2 |
| 310 | 349.0 | 162.0 | 370 | 416.6 | 195.7 | 430 | 484.1 | 230.4 |
| 312 | 351.3 | 163.1 | 372 | 418.8 | 196.8 | 432 | 486.4 | 231.6 |
| 314 | 353.5 | 164.2 | 374 | 421.1 | 198.0 | 434 | 488.6 | 232.8 |
| 316 | 355.8 | 165.3 | 376 | 423.3 | 199.1 | 436 | 490.9 | 233.9 |
| 318 | 358.0 | 166.4 | 378 | 425.6 | 200.3 | 438 | 493.1 | 235.1 |
| 320 | 360.3 | 167.5 | 380 | 427.8 | 201.4 | 440 | 495.4 | 236.3 |
| 322 | 362.5 | 168.6 | 382 | 430.1 | 202.5 | 442 | 497.6 | 237.5 |
| 324 | 364.8 | 169.7 | 384 | 432.3 | 203.7 | 444 | 499.9 | 238.7 |
| 326 | 367.0 | 170.9 | 386 | 434.6 | 204.8 | 446 | 502.1 | 239.8 |
| 328 | 369.3 | 172.0 | 388 | 436.8 | 206.0 | 448 | 504.4 | 241.0 |
| 330 | 371.5 | 173.1 | 390 | 439.1 | 207.1 | 450 | 506.6 | 242.2 |
| 332 | 373.8 | 174.2 | 392 | 441.3 | 208.3 | 452 | 508.9 | 243.4 |
| 334 | 376.0 | 175.3 | 394 | 443.6 | 209.4 | 454 | 511.1 | 244.6 |
| 336 | 378.3 | 176.5 | 396 | 445.9 | 210.6 | 456 | 513.4 | 245.7 |
| 338 | 380.5 | 177.6 | 398 | 448.1 | 211.7 | 458 | 515.6 | 246.9 |
| 340 | 382.8 | 178.7 | 400 | 450.3 | 212.9 | 460 | 517.9 | 248.1 |
|  |  |  |  |  |  | 462 | 520.1 | 249.3 |

**15** *Factors for 10 cc Soxhlet's solution to be used in connection with the Lane-Eynon general volumetric method.*

| TITER | NO SUCROSE INVERT SUGAR | 1 GRAM SUCROSE PER 100 CC INVERT SUGAR | 5 GRAMS SUCROSE PER 100 CC INVERT SUGAR | 10 GRAMS SUCROSE PER 100 CC INVERT SUGAR | 25 GRAMS SUCROSE PER 100 CC INVERT SUGAR | DEXTROSE | LEVULOSE | ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$ | HYDRATED MALTOSE $C_{12}H_{22}O_{11}\cdot H_2O$ | ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$ | HYDRATED LACTOSE $C_{12}H_{22}O_{11}\cdot H_2O$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | 50.5 | 49.9 | 47.6 | 46.1 | 43.4 | 49.1 | 52.2 | 77.2 | 81.3 | 64.9 | 68.3 |
| 16 | 50.6 | 50.0 | 47.6 | 46.1 | 43.4 | 49.2 | 52.3 | 77.1 | 81.2 | 64.8 | 68.2 |
| 17 | 50.7 | 50.1 | 47.6 | 46.1 | 43.4 | 49.3 | 52.3 | 77.0 | 81.1 | 64.8 | 68.2 |
| 18 | 50.8 | 50.1 | 47.6 | 46.1 | 43.3 | 49.3 | 52.4 | 77.0 | 81.0 | 64.7 | 58.1 |
| 19 | 50.8 | 50.2 | 47.6 | 46.1 | 43.3 | 49.4 | 52.5 | 76.9 | 80.9 | 64.7 | 68.1 |
| 20 | 50.9 | 50.2 | 47.6 | 46.1 | 43.2 | 49.5 | 52.5 | 76.8 | 80.8 | 64.6 | 68.0 |
| 21 | 51.0 | 50.2 | 47.6 | 46.1 | 43.2 | 49.5 | 52.6 | 76.7 | 80.7 | 64.6 | 68.0 |
| 22 | 51.0 | 50.3 | 47.6 | 46.1 | 43.1 | 49.6 | 52.7 | 76.6 | 80.6 | 64.6 | 68.0 |
| 23 | 51.1 | 50.3 | 47.6 | 46.1 | 43.0 | 49.7 | 52.7 | 76.5 | 80.5 | 64.5 | 67.9 |
| 24 | 51.2 | 50.3 | 47.6 | 46.1 | 42.9 | 49.8 | 52.8 | 76.4 | 80.4 | 64.5 | 67.9 |
| 25 | 51.2 | 50.4 | 47.6 | 46.0 | 42.8 | 49.8 | 52.8 | 76.4 | 80.4 | 64.5 | 67.9 |
| 26 | 51.3 | 50.4 | 47.6 | 46.0 | 42.8 | 49.9 | 52.9 | 76.3 | 80.3 | 64.5 | 67.9 |
| 27 | 51.4 | 50.4 | 47.6 | 46.0 | 42.7 | 49.9 | 52.9 | 76.2 | 80.2 | 64.4 | 67.8 |
| 28 | 51.4 | 50.5 | 47.7 | 46.0 | 42.7 | 50.0 | 53.0 | 76.1 | 80.1 | 64.4 | 67.8 |
| 29 | 51.5 | 50.5 | 47.7 | 46.0 | 42.6 | 50.0 | 53.1 | 76.0 | 80.0 | 64.4 | 57.8 |
| 30 | 51.5 | 50.5 | 47.7 | 46.0 | 42.5 | 50.1 | 53.2 | 76.0 | 80.0 | 64.4 | 67.8 |
| 31 | 51.6 | 50.6 | 47.7 | 45.9 | 42.5 | 50.2 | 53.2 | 75.9 | 79.9 | 64.4 | 67.8 |
| 32 | 51.6 | 50.6 | 47.7 | 45.9 | 42.4 | 50.2 | 53.3 | 75.9 | 79.9 | 64.4 | 67.8 |
| 33 | 51.7 | 50.6 | 47.7 | 45.9 | 42.3 | 50.3 | 53.3 | 75.8 | 79.8 | 64.4 | 67.8 |
| 34 | 51.7 | 50.6 | 47.7 | 45.8 | 42.2 | 50.3 | 53.4 | 75.8 | 79.8 | 64.4 | 67.9 |
| 35 | 51.8 | 50.7 | 47.7 | 45.8 | 42.2 | 50.4 | 53.4 | 75.7 | 79.7 | 64.5 | 67.9 |
| 36 | 51.8 | 50.7 | 47.7 | 45.8 | 42.1 | 50.4 | 53.5 | 75.6 | 79.6 | 64.5 | 67.9 |
| 37 | 51.9 | 50.7 | 47.7 | 45.7 | 42.0 | 50.5 | 53.5 | 75.6 | 79.6 | 64.5 | 67.9 |
| 38 | 51.9 | 50.7 | 47.7 | 45.7 | 42.0 | 50.5 | 53.6 | 75.5 | 79.5 | 63.5 | 67.9 |
| 39 | 52.0 | 50.8 | 47.7 | 45.7 | 41.9 | 50.6 | 53.6 | 75.5 | 79.5 | 64.5 | 67.9 |
| 40 | 52.0 | 50.8 | 47.7 | 45.6 | 41.8 | 50.6 | 53.6 | 75.4 | 79.4 | 64.5 | 67.9 |
| 41 | 52.1 | 50.8 | 47.7 | 45.6 | 41.8 | 50.7 | 53.7 | 75.4 | 79.4 | 64.6 | 68.0 |
| 42 | 52.1 | 50.8 | 47.7 | 45.6 | 41.7 | 50.7 | 53.7 | 75.3 | 79.3 | 64.6 | 68.0 |
| 43 | 52.2 | 50.8 | 47.7 | 45.5 | 41.6 | 50.8 | 53.8 | 75.3 | 79.3 | 64.6 | 68.0 |
| 44 | 52.2 | 50.9 | 47.7 | 45.5 | 41.5 | 50.8 | 53.8 | 75.2 | 79.2 | 64.6 | 68.0 |
| 45 | 52.3 | 50.9 | 47.7 | 45.4 | 41.4 | 50.9 | 53.9 | 75.2 | 79.2 | 64.7 | 68.1 |
| 46 | 52.3 | 50.9 | 47.7 | 45.4 | 41.4 | 50.9 | 53.9 | 75.1 | 79.1 | 64.7 | 68.1 |
| 47 | 52.4 | 50.9 | 47.7 | 45.3 | 41.3 | 51.0 | 53.9 | 75.1 | 79.1 | 64.8 | 68.2 |
| 48 | 52.4 | 50.9 | 47.7 | 45.3 | 41.2 | 51.0 | 54.0 | 75.1 | 79.1 | 64.8 | 68.2 |
| 49 | 52.5 | 51.0 | 47.7 | 45.2 | 41.1 | 51.0 | 54.0 | 75.0 | 79.0 | 64.8 | 68.2 |
| 50 | 52.5 | 51.0 | 47.7 | 45.2 | 41.0 | 51.1 | 54.0 | 75.0 | 79.0 | 64.9 | 68.3 |

*Factors for 25 cc Soxhlet's solution to be used in connection with the Lane-Eynon general volumetric method.* 16

| TITER | NO SUCROSE INVERT SUGAR | 1 GRAM SUCROSE PER 100 CC INVERT SUGAR | DEXTROSE | LEVULOSE | ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$ | HYDRATED MALTOSE $C_{12}H_{22}O_{11}\cdot H_2O$ | ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$ | HYDRATED LACTOSE $C_{12}H_{22}O_{11}\cdot H_2O$ |
|---|---|---|---|---|---|---|---|---|
| 15 | 123.6 | 122.6 | 120.2 | 127.4 | 197.8 | 208.2 | 163.9 | 172.5 |
| 16 | 123.6 | 122.7 | 120.2 | 127.4 | 197.4 | 207.8 | 163.5 | 172.1 |
| 17 | 123.6 | 122.7 | 120.2 | 127.5 | 197.0 | 207.4 | 163.1 | 171.7 |
| 18 | 123.7 | 122.7 | 120.2 | 127.5 | 196.7 | 207.1 | 162.8 | 171.4 |
| 19 | 123.7 | 122.8 | 120.3 | 127.6 | 196.5 | 206.8 | 162.5 | 171.1 |
| 20 | 123.8 | 122.8 | 120.3 | 127.6 | 196.2 | 206.5 | 162.3 | 170.9 |
| 21 | 123.8 | 122.8 | 120.3 | 127.7 | 195.8 | 206.1 | 162.0 | 170.6 |
| 22 | 123.9 | 122.9 | 120.4 | 127.7 | 195.5 | 205.8 | 161.8 | 170.4 |
| 23 | 123.9 | 122.9 | 120.4 | 127.8 | 195.1 | 205.4 | 161.6 | 170.2 |
| 24 | 124.0 | 122.9 | 120.5 | 127.8 | 194.8 | 205.1 | 161.5 | 170.0 |
| 25 | 124.0 | 123.0 | 120.5 | 127.9 | 194.5 | 204.8 | 161.4 | 169.9 |
| 26 | 124.1 | 123.0 | 120.6 | 127.9 | 194.2 | 204.4 | 161.2 | 169.7 |
| 27 | 124.1 | 123.0 | 120.6 | 128.0 | 193.9 | 204.1 | 161.0 | 169.5 |
| 28 | 124.2 | 123.1 | 120.7 | 128.0 | 193.6 | 203.8 | 160.8 | 169.3 |
| 29 | 124.2 | 123.1 | 120.7 | 128.1 | 193.3 | 203.5 | 160.7 | 169.2 |
| 30 | 124.3 | 123.1 | 120.8 | 128.1 | 193.0 | 203.2 | 160.6 | 169.0 |
| 31 | 124.3 | 123.2 | 120.8 | 128.1 | 192.8 | 202.9 | 160.5 | 168.9 |
| 32 | 124.4 | 123.2 | 120.8 | 128.2 | 192.5 | 202.6 | 160.4 | 168.8 |
| 33 | 124.4 | 123.2 | 120.9 | 128.2 | 192.2 | 202.3 | 160.2 | 168.6 |
| 34 | 124.5 | 123.3 | 120.9 | 128.3 | 191.9 | 202.0 | 160.1 | 168.5 |
| 35 | 124.5 | 123.3 | 121.0 | 128.3 | 191.7 | 201.8 | 160.0 | 168.4 |
| 36 | 124.6 | 123.3 | 121.0 | 128.4 | 191.4 | 201.5 | 159.8 | 168.2 |
| 37 | 125.6 | 123.4 | 121.1 | 128.4 | 191.2 | 201.2 | 159.7 | 168.1 |
| 38 | 124.7 | 123.4 | 121.2 | 128.5 | 191.0 | 201.0 | 159.6 | 168.0 |
| 39 | 124.7 | 123.4 | 121.2 | 128.5 | 190.8 | 200.8 | 159.5 | 167.9 |
| 40 | 124.8 | 123.4 | 121.2 | 128.6 | 190.5 | 200.5 | 159.4 | 167.8 |
| 41 | 124.8 | 123.5 | 121.3 | 128.6 | 190.3 | 200.3 | 159.3 | 167.7 |
| 42 | 124.9 | 123.5 | 121.4 | 128.6 | 190.1 | 200.1 | 159.2 | 167.6 |
| 43 | 124.9 | 123.5 | 121.4 | 128.7 | 189.8 | 199.8 | 159.2 | 167.6 |
| 44 | 125.0 | 123.6 | 121.5 | 128.7 | 189.6 | 199.6 | 159.1 | 167.5 |
| 45 | 125.0 | 123.6 | 121.5 | 128.8 | 189.4 | 199.4 | 159.0 | 167.4 |
| 46 | 125.1 | 123.6 | 121.6 | 128.8 | 189.2 | 199.2 | 159.0 | 167.4 |
| 47 | 125.1 | 123.7 | 121.6 | 128.9 | 189.0 | 199.0 | 158.9 | 167.3 |
| 48 | 125.2 | 123.7 | 121.7 | 128.9 | 188.9 | 198.9 | 158.8 | 167.2 |
| 49 | 125.2 | 123.7 | 121.7 | 129.0 | 188.8 | 198.7 | 158.8 | 167.2 |
| 50 | 125.3 | 123.8 | 121.8 | 129.0 | 188.7 | 198.6 | 158.7 | 167.1 |

## 17 *Quisumbing and Thomas table for calculating dextrose, levulose, invert sugar, lactose, and maltose.*

(Expressed in milligrams.)

| COPPER (Cu) | CUPROUS OXIDE $(Cu_2O)$ | DEXTROSE (*d*-GLUCOSE) | LEVULOSE (*d*-FRUCTOSE) | INVERT SUGAR | LACTOSE | | MALTOSE | |
|---|---|---|---|---|---|---|---|---|
| | | | | | $C_{12}H_{22}O_{11}$ | $\cdot C_{12}H_{22}O_{11} \cdot H_2O \cdot$ | $C_{12}H_{22}O_{11}$ | $\cdot C_{12}H_{22}O_{11} \cdot H_2O$ |
| 10 | 11.1 | 4.8 | 5.3 | 5.0 | 7.7 | 8.1 | 9.4 | 9.9 |
| 20 | 22.5 | 9.5 | 10.5 | 10.1 | 15.5 | 16.3 | 18.8 | 19.8 |
| 30 | 33.8 | 14.3 | 15.8 | 15.2 | 23.2 | 24.4 | 28.2 | 29.7 |
| 40 | 45.0 | 19.1 | 21.2 | 20.3 | 30.9 | 32.5 | 37.6 | 39.6 |
| 50 | 56.3 | 24.0 | 26.5 | 25.4 | 38.7 | 40.7 | 47.0 | 49.5 |
| 60 | 67.6 | 28.9 | 31.9 | 30.6 | 46.4 | 48.8 | 56.4 | 59.4 |
| 70 | 78.8 | 33.7 | 37.2 | 35.7 | 54.0 | 56.9 | 65.8 | 69.3 |
| 80 | 90.1 | 38.7 | 42.6 | 40.9 | 61.7 | 65.0 | 75.2 | 79.2 |
| 90 | 101.3 | 43.6 | 48.0 | 46.1 | 69.5 | 73.2 | 84.6 | 89.1 |
| 100 | 112.6 | 48.6 | 53.4 | 51.3 | 77.2 | 81.3 | 94.0 | 99.0 |
| 110 | 123.8 | 53.5 | 58.8 | 56.5 | 85.0 | 89.5 | 103.4 | 108.9 |
| 120 | 135.1 | 58.5 | 64.3 | 61.8 | 92.7 | 97.0 | 112.8 | 118.8 |
| 130 | 146.4 | 63.5 | 70.7 | 67.0 | 100.4 | 105.7 | 122.2 | 128.7 |
| 140 | 157.6 | 68.6 | 75.2 | 72.3 | 108.2 | 113.9 | 131.6 | 138.6 |
| 150 | 168.9 | 73.7 | 80.7 | 77.6 | 116.0 | 122.0 | 141.0 | 148.5 |
| 160 | 180.1 | 78.8 | 86.2 | 82.9 | 123.7 | 130.1 | 150.4 | 158.4 |
| 170 | 191.4 | 83.9 | 91.7 | 88.3 | 131.4 | 138.3 | 159.8 | 168.3 |
| 180 | 202.6 | 89.1 | 97.2 | 93.7 | 139.1 | 146.4 | 169.2 | 178.2 |
| 190 | 213.9 | 94.2 | 102.8 | 99.1 | 146.9 | 154.6 | 178.8 | 188.1 |
| 200 | 225.2 | 99.4 | 108.4 | 104.4 | 154.6 | 162.7 | 188.2 | 198.0 |
| 210 | 236.4 | 104.6 | 114.0 | 109.8 | 162.3 | 170.9 | 197.6 | 207.9 |
| 220 | 247.7 | 109.9 | 119.6 | 115.2 | 170.0 | 179.0 | 207.0 | 217.8 |
| 230 | 258.9 | 115.1 | 125.2 | 120.6 | 177.8 | 187.2 | 216.4 | 227.7 |
| 240 | 270.2 | 120.4 | 130.8 | 126.1 | 185.5 | 195.3 | 225.8 | 237.6 |
| 250 | 281.5 | 125.7 | 136.4 | 131.6 | 193.2 | 203.4 | 235.2 | 247.5 |
| 260 | 292.7 | 131.0 | 142.1 | 137.1 | 201.0 | 211.6 | 244.6 | 257.4 |
| 270 | 304.0 | 136.4 | 147.8 | 142.6 | 208.8 | 219.8 | 254.0 | 267.3 |
| 280 | 315.2 | 141.7 | 153.5 | 148.2 | 216.5 | 227.9 | 263.4 | 277.2 |
| 290 | 326.5 | 147.1 | 159.2 | 153.7 | 224.2 | 236.0 | 272.8 | 287.1 |
| 300 | 337.8 | 152.6 | 165.0 | 159.3 | 232.0 | 244.2 | 282.2 | 297.0 |
| 310 | 349.0 | 158.0 | 170.7 | 164.9 | 239.7 | 252.3 | 291.6 | 306.9 |
| 320 | 360.3 | 163.5 | 176.5 | 170.5 | 247.5 | 260.5 | 301.0 | 316.8 |
| 330 | 371.5 | 168.9 | 182.3 | 176.1 | 255.3 | 268.7 | 310.4 | 326.7 |
| 340 | 382.8 | 174.5 | 188.1 | 181.8 | 263.0 | 276.8 | 319.8 | 336.6 |
| 350 | 394.0 | 180.0 | 193.9 | 187.4 | 270.7 | 285.0 | 329.2 | 346.5 |
| 360 | 405.3 | 185.5 | 199.7 | 193.1 | 278.4 | 293.1 | 338.6 | 356.4 |
| 370 | 416.6 | 191.1 | 205.5 | 198.8 | 286.2 | 301.3 | 348.0 | 366.3 |
| 380 | 427.8 | 196.7 | 211.4 | 204.5 | 293.9 | 309.4 | 357.4 | 376.2 |
| 390 | 439.1 | 202.3 | 217.3 | 210.2 | 301.6 | 317.5 | 366.8 | 386.1 |
| 400 | 450.3 | 208.0 | 223.2 | 216.0 | 309.4 | 325.7 | 376.2 | 396.0 |
| 410 | 461.6 | 213.7 | 229.1 | 221.8 | 317.1 | 333.8 | 385.6 | 405.9 |
| 420 | 472.9 | 219.4 | 235.0 | 227.6 | 324.9 | 342.0 | 395.0 | 415.8 |
| 430 | 484.1 | 325.1 | 240.9 | 233.4 | 332.6 | 350.1 | 404.4 | 425.7 |
| 440 | 495.4 | 230.8 | 246.9 | 239.2 | 340.4 | 358.3 | 413.8 | 435.6 |
| 450 | 506.6 | 236.6 | 252.9 | 245.0 | 348.1 | 366.4 | 423.2 | 445.5 |
| 460 | 517.9 | 242.4 | 258.9 | 250.9 | 355.9 | 374.6 | 432.6 | 455.4 |
| 470 | 529.1 | 248.1 | 264.9 | 256.8 | 363.6 | 382.7 | 442.0 | 465.3 |
| 480 | 540.4 | 250.8 | 270.9 | 262.7 | 371.3 | 390.9 | 451.4 | 475.2 |

## *Kröber's table for the determination of pentoses and pentosans.* 18

(Expressed in grams.)

| FURFURAL PHLOROGLUCIDE | FURFURAL | ARABINOSE | ARABAN | XYLOSE | XYLAN | PENTOSE | PENTOSAN |
|---|---|---|---|---|---|---|---|
| 0.030 | 0.0182 | 0.0391 | 0.0344 | 0.0324 | 0.0285 | 0.0358 | 0.0315 |
| 0.032 | 0.0193 | 0.0413 | 0.0363 | 0.0342 | 0.0301 | 0.0378 | 0.0333 |
| 0.034 | 0.0203 | 0.0435 | 0.0383 | 0.0361 | 0.0317 | 0.0398 | 0.0350 |
| 0.036 | 0.0214 | 0.0457 | 0.0402 | 0.0379 | 0.0334 | 0.0418 | 0.0368 |
| 0.038 | 0.0224 | 0.0479 | 0.0422 | 0.0398 | 0.0350 | 0.0439 | 0.0386 |
| 0.040 | 0.0235 | 0.0501 | 0.0441 | 0.0416 | 0.0366 | 0.0459 | 0.0404 |
| 0.042 | 0.0245 | 0.0523 | 0.0460 | 0.0434 | 0.0382 | 0.0479 | 0.0422 |
| 0.044 | 0.0255 | 0.0545 | 0.0480 | 0.0452 | 0.0398 | 0.0499 | 0.0440 |
| 0.046 | 0.0266 | 0.0567 | 0.0499 | 0.0471 | 0.0414 | 0.0519 | 0.0457 |
| 0.048 | 0.0276 | 0.0589 | 0.0519 | 0.0489 | 0.0430 | 0.0539 | 0.0475 |
| 0.050 | 0.0286 | 0.0611 | 0.0538 | 0.0507 | 0.0446 | 0.0559 | 0.0492 |
| 0.052 | 0.0297 | 0.0633 | 0.0557 | 0.0525 | 0.0462 | 0.0579 | 0.0510 |
| 0.054 | 0.0307 | 0.0655 | 0.0576 | 0.0543 | 0.0478 | 0.0599 | 0.0528 |
| 0.056 | 0.0318 | 0.0677 | 0.0596 | 0.0562 | 0.0494 | 0.0620 | 0.0546 |
| 0.058 | 0.0328 | 0.0699 | 0.0615 | 0.0580 | 0.0510 | 0.0640 | 0.0564 |
| 0.060 | 0.0338 | 0.0721 | 0.0634 | 0.0598 | 0.0526 | 0.0660 | 0.0581 |
| 0.062 | 0.0349 | 0.0743 | 0.0653 | 0.0616 | 0.0542 | 0.0680 | 0.0599 |
| 0.064 | 0.0359 | 0.0765 | 0.0673 | 0.0635 | 0.0558 | 0.0700 | 0.0617 |
| 0.066 | 0.0370 | 0.0787 | 0.0692 | 0.0653 | 0.0575 | 0.0720 | 0.0634 |
| 0.068 | 0.0380 | 0.0809 | 0.0712 | 0.0672 | 0.0591 | 0.0741 | 0.0652 |
| 0.070 | 0.0390 | 0.0831 | 0.0731 | 0.0690 | 0.0607 | 0.0761 | 0.0670 |
| 0.072 | 0.0401 | 0.0853 | 0.0750 | 0.0708 | 0.0623 | 0.0781 | 0.0688 |
| 0.074 | 0.0411 | 0.0875 | 0.0770 | 0.0726 | 0.0639 | 0.0801 | 0.0706 |
| 0.076 | 0.0422 | 0.0897 | 0.0789 | 0.0745 | 0.0655 | 0.0821 | 0.0722 |
| 0.078 | 0.0432 | 0.0919 | 0.0809 | 0.0763 | 0.0671 | 0.0841 | 0.0740 |
| 0.080 | 0.0442 | 0.0941 | 0.0828 | 0.0781 | 0.0687 | 0.0861 | 0.0758 |
| 0.082 | 0.0453 | 0.0963 | 0.0847 | 0.0799 | 0.0703 | 0.0881 | 0.0776 |
| 0.084 | 0.0463 | 0.0985 | 0.0867 | 0.0817 | 0.0719 | 0.0901 | 0.0794 |
| 0.086 | 0.0474 | 0.1007 | 0.0886 | 0.0836 | 0.0735 | 0.0922 | 0.0812 |
| 0.088 | 0.0484 | 0.1029 | 0.0906 | 0.0854 | 0.0751 | 0.0942 | 0.0830 |
| 0.090 | 0.0494 | 0.1051 | 0.0925 | 0.0872 | 0.0767 | 0.0962 | 0.0847 |
| 0.092 | 0.0505 | 0.1073 | 0.0944 | 0.0890 | 0.0783 | 0.0982 | 0.0865 |
| 0.094 | 0.0515 | 0.1095 | 0.0964 | 0.0909 | 0.0800 | 0.1002 | 0.0883 |
| 0.096 | 0.0525 | 0.1117 | 0.0983 | 0.0927 | 0.0816 | 0.1022 | 0.0899 |
| 0.098 | 0.0536 | 0.1139 | 0.1003 | 0.0946 | 0.0832 | 0.1043 | 0.0917 |
| 0.100 | 0.0546 | 0.1161 | 0.1022 | 0.0964 | 0.0848 | 0.1063 | 0.0935 |
| 0.102 | 0.0557 | 0.1182 | 0.1041 | 0.0982 | 0.0864 | 0.1083 | 0.0953 |
| 0.104 | 0.0567 | 0.1204 | 0.1060 | 0.1000 | 0.0880 | 0.1103 | 0.0971 |
| 0.106 | 0.0577 | 0.1226 | 0.1080 | 0.1019 | 0.0896 | 0.1123 | 0.0988 |
| 0.108 | 0.0588 | 0.1248 | 0.1099 | 1.1037 | 0.0912 | 0.1143 | 0.1006 |
| 0.110 | 0.0598 | 0.1270 | 0.1118 | 0.1055 | 0.0928 | 0.1163 | 0.1023 |
| 0.112 | 0.0608 | 0.1292 | 0.1137 | 0.1073 | 0.0944 | 0.1183 | 0.1041 |
| 0.114 | 0.0619 | 0.1314 | 0.1156 | 0.1091 | 0.0960 | 0.1203 | 0.1059 |
| 0.116 | 0.0629 | 0.1336 | 0.1176 | 0.1110 | 0.0976 | 0.1223 | 0.1076 |
| 0.118 | 0.0640 | 0.1358 | 0.1195 | 0.1128 | 0.0992 | 0.1243 | 0.1094 |

18

### *Kröber's table.*—Continued.

(Expressed in grams.)

| FURFURAL PHLOROGLUCIDE | FURFURAL | ARABINOSE | ARABAN | XYLOSE | XYLAN | PENTOSE | PENTOSAN |
|---|---|---|---|---|---|---|---|
| 0.120 | 0.0650 | 0.1380 | 0.1214 | 0.1146 | 0.1008 | 0.1263 | 0.1111 |
| 0.122 | 0.0660 | 0.1402 | 0.1233 | 0.1164 | 0.1024 | 0.1283 | 0.1129 |
| 0.124 | 0.0671 | 0.1424 | 0.1253 | 0.1182 | 0.1040 | 0.1303 | 0.1147 |
| 0.126 | 0.0681 | 0.1446 | 0.1272 | 0.1201 | 0.1057 | 0.1324 | 0.1165 |
| 0.128 | 0.0691 | 0.1468 | 0.1292 | 0.1219 | 0.1073 | 0.1344 | 0.1183 |
| 0.130 | 0.0702 | 0.1490 | 0.1311 | 0.1237 | 0.1089 | 0.1364 | 0.1201 |
| 0.132 | 0.0712 | 0.1512 | 0.1330 | 0.1255 | 0.1105 | 0.1384 | 0.1219 |
| 0.134 | 0.0723 | 0.1534 | 0.1350 | 0.1273 | 0.1121 | 0.1404 | 0.1236 |
| 0.136 | 0.0733 | 0.1556 | 0.1369 | 0.1292 | 0.1137 | 0.1424 | 0.1253 |
| 0.138 | 0.0743 | 0.1578 | 0.1389 | 0.1310 | 0.1153 | 0.1444 | 0.1271 |
| 0.140 | 0.0754 | 0.1600 | 0.1408 | 0.1328 | 0.1169 | 0.1464 | 0.1288 |
| 0.142 | 0.0764 | 0.1622 | 0.1427 | 0.1346 | 0.1185 | 0.1484 | 0.1306 |
| 0.144 | 0.0774 | 0.1644 | 0.1447 | 0.1364 | 0.1201 | 0.1504 | 0.1324 |
| 0.146 | 0.0785 | 0.1666 | 0.1466 | 0.1383 | 0.1217 | 0.1525 | 0.1342 |
| 0.148 | 0.0795 | 0.1688 | 0.1486 | 0.1401 | 0.1233 | 0.1545 | 0.1360 |
| 0.150 | 0.0805 | 0.1710 | 0.1505 | 0.1419 | 0.1249 | 0.1565 | 0.1377 |
| 0.152 | 0.0816 | 0.1732 | 0.1524 | 0.1437 | 0.1265 | 0.1585 | 0.1395 |
| 0.154 | 0.0826 | 0.1754 | 0.1544 | 0.1455 | 0.1281 | 0.1605 | 0.1413 |
| 0.156 | 0.0837 | 0.1776 | 0.1563 | 0.1474 | 0.1297 | 0.1625 | 0.1430 |
| 0.158 | 0.0847 | 0.1798 | 0.1583 | 0.1492 | 0.1313 | 0.1645 | 0.1448 |
| 0.160 | 0.0857 | 0.1820 | 0.1602 | 0.1510 | 0.1329 | 0.1665 | 0.1465 |
| 0.162 | 0.0868 | 0.1842 | 0.1621 | 0.1528 | 0.1345 | 0.1685 | 0.1483 |
| 0.164 | 0.0878 | 0.1864 | 0.1640 | 0.1546 | 0.1361 | 0.1705 | 0.1501 |
| 0.166 | 0.0888 | 0.1886 | 0.1660 | 0.1565 | 0.1377 | 0.1726 | 0.1519 |
| 0.168 | 0.0899 | 0.1908 | 0.1679 | 0.1583 | 0.1393 | 0.1746 | 0.1537 |
| 0.170 | 0.0909 | 0.1930 | 0.1698 | 0.1601 | 0.1409 | 0.1766 | 0.1554 |
| 0.172 | 0.0920 | 0.1952 | 0.1717 | 0.1619 | 0.1425 | 0.1786 | 0.1572 |
| 0.174 | 0.0930 | 0.1974 | 0.1736 | 0.1637 | 0.1441 | 0.1806 | 0.1590 |
| 0.176 | 0.0940 | 0.1996 | 0.1756 | 0.1656 | 0.1457 | 0.1826 | 0.1607 |
| 0.178 | 0.0951 | 0.2018 | 0.1775 | 0.1674 | 0.1473 | 0.1846 | 0.1625 |
| 0.180 | 0.0961 | 0.2039 | 0.1794 | 0.1692 | 0.1489 | 0.1866 | 0.1642 |
| 0.182 | 0.0971 | 0.2061 | 0.1813 | 0.1710 | 0.1505 | 0.1886 | 0.1660 |
| 0.184 | 0.0982 | 0.2082 | 0.1832 | 0.1728 | 0.1521 | 0.1906 | 0.1678 |
| 0.186 | 0.0992 | 0.2104 | 0.1851 | 0.1747 | 0.1537 | 0.1926 | 0.1695 |
| 0.188 | 0.1003 | 0.2126 | 0.1870 | 0.1765 | 0.1553 | 0.1946 | 0.1712 |
| 0.190 | 0.1013 | 0.2147 | 0.1889 | 0.1783 | 0.1569 | 0.1965 | 0.1729 |
| 0.192 | 0.1023 | 0.2168 | 0.1908 | 0.1801 | 0.1585 | 0.1985 | 0.1747 |
| 0.194 | 0.1034 | 0.2190 | 0.1927 | 0.1819 | 0.1601 | 0.2005 | 0.1764 |
| 0.196 | 0.1044 | 0.2212 | 0.1946 | 0.1838 | 0.1617 | 0.2025 | 0.1782 |
| 0.198 | 0.1054 | 0.2233 | 0.1965 | 0.1856 | 0.1633 | 0.2045 | 0.1800 |
| 0.200 | 0.1065 | 0.2255 | 0.1984 | 0.1874 | 0.1649 | 0.2065 | 0.1817 |
| 0.202 | 0.1075 | 0.2276 | 0.2003 | 0.1892 | 0.1665 | 0.2085 | 0.1835 |
| 0.204 | 0.1085 | 0.2298 | 0.2022 | 0.1910 | 0.1681 | 0.2105 | 0.1853 |
| 0.206 | 0.1096 | 0.2320 | 0.2041 | 0.1929 | 0.1697 | 0.2125 | 0.1869 |
| 0.208 | 0.1106 | 0.2341 | 0.2060 | 0.1947 | 0.1713 | 0.2144 | 0.1887 |

## *Kröber's table.*—Continued.

(Expressed in grams.)

| FURFURAL PHLOROGLUCIDE | FUFURAL | ARABINOSE | ARABAN | XYLOSE | XYLAN | PENTOSE | PENTOSAN |
|---|---|---|---|---|---|---|---|
| 0.210 | 0.1116 | 0.2363 | 0.2079 | 0.1965 | 0.1729 | 0.2164 | 0.1904 |
| 0.212 | 0.1127 | 0.2384 | 0.2098 | 0.1984 | 0.1745 | 0.2184 | 0.1922 |
| 0.214 | 0.1137 | 0.2406 | 0.2117 | 0.2002 | 0.1761 | 0.2204 | 0.1940 |
| 0.216 | 0.1147 | 0.2428 | 0.2136 | 0.2020 | 0.1778 | 0.2224 | 0.1957 |
| 0.218 | 0.1158 | 0.2449 | 0.2155 | 0.2038 | 0.1794 | 0.2244 | 0.1974 |
| 0.220 | 0.1168 | 0.2471 | 0.2174 | 0.2057 | 0.1810 | 0.2264 | 0.1992 |
| 0.222 | 0.1178 | 0.2492 | 0.2193 | 0.2075 | 0.1826 | 0.2284 | 0.2010 |
| 0.224 | 0.1189 | 0.2514 | 0.2212 | 0.2093 | 0.1842 | 0.2304 | 0.2028 |
| 0.226 | 0.1199 | 0.2536 | 0.2232 | 0.2111 | 0.1858 | 0.2324 | 0.2046 |
| 0.228 | 0.1209 | 0.2557 | 0.2251 | 0.2130 | 0.1874 | 0.2344 | 0.2063 |
| 0.230 | 0.1220 | 0.2579 | 0.2270 | 0.2148 | 0.1890 | 0.2364 | 0.2081 |
| 0.232 | 0.1230 | 0.2600 | 0.2289 | 0.2166 | 0.1906 | 0.2383 | 0.2097 |
| 0.234 | 0.1240 | 0.2622 | 0.2308 | 0.2184 | 0.1922 | 0.2403 | 0.2115 |
| 0.236 | 0.1251 | 0.2644 | 0.2327 | 0.2202 | 0.1938 | 0.2423 | 0.2132 |
| 0.238 | 0.1261 | 0.2665 | 0.2346 | 0.2220 | 0.1954 | 0.2443 | 0.2150 |
| 0.240 | 0.1271 | 0.2687 | 0.2365 | 0.2239 | 0.1970 | 0.2463 | 0.2168 |
| 0.242 | 0.1281 | 0.2708 | 0.2384 | 0.2257 | 0.1986 | 0.2483 | 0.2185 |
| 0.244 | 0.1292 | 0.2730 | 0.2403 | 0.2275 | 0.2002 | 0.2503 | 0.2203 |
| 0.246 | 0.1302 | 0.2752 | 0.2422 | 0.2293 | 0.2018 | 0.2523 | 0.2220 |
| 0.248 | 0.1312 | 0.2773 | 0.2441 | 0.2311 | 0.2034 | 0.2543 | 0.2238 |
| 0.250 | 0.1323 | 0.2795 | 0.2460 | 0.2330 | 0.2050 | 0.2563 | 0.2256 |
| 0.252 | 0.1333 | 0.1816 | 0.2479 | 0.2348 | 0.2066 | 0.2582 | 0.2272 |
| 0.254 | 0.1343 | 0.2838 | 0.2498 | 0.2366 | 0.2082 | 0.2602 | 0.2290 |
| 0.256 | 0.1354 | 0.2860 | 0.2517 | 0.2384 | 0.2098 | 0.2622 | 0.2307 |
| 0.258 | 0.1364 | 0.2881 | 0.2536 | 0.2402 | 0.2114 | 0.2642 | 0.2325 |
| 0.260 | 0.1374 | 0.2903 | 0.2555 | 0.2420 | 0.2130 | 0.2662 | 0.2342 |
| 0.262 | 0.1385 | 0.2924 | 0.2574 | 0.2438 | 0.2146 | 0.2681 | 0.2359 |
| 0.264 | 0.1395 | 0.2946 | 0.2593 | 0.2456 | 0.2162 | 0.2701 | 0.2377 |
| 0.266 | 0.1405 | 0.2968 | 0.2612 | 0.2474 | 0.2178 | 0.2721 | 0.2394 |
| 0.268 | 0.1416 | 0.2989 | 0.2631 | 0.2492 | 0.2194 | 0.2741 | 0.2412 |
| 0.270 | 0.1426 | 0.3011 | 0.2650 | 0.2511 | 0.2210 | 0.2761 | 0.2429 |
| 0.272 | 0.1436 | 0.3032 | 0.2669 | 0.2529 | 0.2226 | 0.2781 | 0.2447 |
| 0.274 | 0.1447 | 0.3054 | 0.2688 | 0.2547 | 0.2242 | 0.2801 | 0.2465 |
| 0.276 | 0.1457 | 0.3076 | 0.2707 | 0.2565 | 0.2258 | 0.2821 | 0.2482 |
| 0.278 | 0.1467 | 0.3097 | 0.2726 | 0.2583 | 0.2274 | 0.2840 | 0.2499 |
| 0.280 | 0.1478 | 0.3119 | 0.2745 | 0.2602 | 0.2290 | 0.2861 | 0.2517 |
| 0.282 | 0.1488 | 0.3140 | 0.2764 | 0.2620 | 0.2306 | 0.2880 | 0.2534 |
| 0.284 | 0.1498 | 0.3162 | 0.2783 | 0.2638 | 0.2322 | 0.2900 | 0.2552 |
| 0.286 | 0.1509 | 0.3184 | 0.2802 | 0.2656 | 0.2338 | 0.2920 | 0.2570 |
| 0.288 | 0.1519 | 0.3205 | 0.2821 | 0.2674 | 0.2354 | 0.2940 | 0.2587 |
| 0.290 | 0.1529 | 0.3227 | 0.2840 | 0.2693 | 0.2370 | 0.2960 | 0.2605 |
| 0.292 | 0.1540 | 0.3248 | 0.2859 | 0.2711 | 0.2386 | 0.2980 | 0.2622 |
| 0.294 | 0.1550 | 0.3270 | 0.2878 | 0.2729 | 0.2402 | 0.3000 | 0.2640 |
| 0.296 | 0.1560 | 0.3292 | 0.2897 | 0.2747 | 0.2418 | 0.3020 | 0.2658 |
| 0.298 | 0.1571 | 0.3313 | 0.2916 | 0.2765 | 0.2434 | 0.3040 | 0.2675 |
| 0.300 | 0.1581 | 0.3335 | 0.2935 | 0.2784 | 0.2450 | 0.3060 | 0.2693 |

19 *Relation between specific gravity at 15.56/15.56°C. and alcohol content of mixtures of ethyl alcohol and water.*[1]

| SP. GR. 15.56/15.56° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC | SP. GR. 15.56/15.56° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC | SP. GR. 15.56/15.56° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.00000 | 0.0 | *0.00* | 0.00 | 0.99350 | 4.5 | *3.60* | 3.57 | 0.98779 | 9.0 | *7.23* | 7.1 |
| 0.99985 | 0.1 | *0.08* | 0.08 | 0.99336 | 4.6 | *3.68* | 3.65 | 0.98767 | 9.1 | *7.31* | 7.2 |
| 0.99970 | 0.2 | *0.16* | 0.16 | 0.99323 | 4.7 | *3.76* | 3.73 | 0.98755 | 9.2 | *7.40* | 7.3 |
| 0.99955 | 0.3 | *0.24* | 0.24 | 0.99309 | 4.8 | *3.84* | 3.81 | 0.98743 | 9.3 | *7.48* | 7.3 |
| 0.99940 | 0.4 | *0.32* | 0.32 | 0.99296 | 4.9 | *3.92* | 3.89 | 0.98731 | 9.4 | *7.56* | 7.4 |
| 0.99925 | 0.5 | *0.40* | 0.40 | 0.99282 | 5.0 | *4.00* | 3.97 | 0.98720 | 9.5 | *7.64* | 7.5 |
| 0.99910 | 0.6 | *0.48* | 0.48 | 0.99269 | 5.1 | *4.08* | 4.04 | 0.98708 | 9.6 | *7.72* | 7.6 |
| 0.99895 | 0.7 | *0.56* | 0.56 | 0.99255 | 5.2 | *4.16* | 4.12 | 0.98696 | 9.7 | *7.80* | 7.6 |
| 0.99880 | 0.8 | *0.64* | 0.63 | 0.99242 | 5.3 | *4.24* | 4.20 | 0.98684 | 9.8 | *7.88* | 7.7 |
| 0.99865 | 0.9 | *0.72* | 0.71 | 0.99228 | 5.4 | *4.32* | 4.28 | 0.98672 | 9.9 | *7.97* | 7.8 |
| 0.99850 | 1.0 | *0.80* | 0.79 | 0.99215 | 5.5 | *4.40* | 4.36 | 0.98661 | 10.0 | *8.05* | 7.9 |
| 0.99835 | 1.1 | *0.87* | 0.87 | 0.99202 | 5.6 | *4.48* | 4.44 | 0.98649 | 10.1 | *8.13* | 8.0 |
| 0.99820 | 1.2 | *0.95* | 0.95 | 0.99189 | 5.7 | *4.56* | 4.52 | 0.98637 | 10.2 | *8.21* | 8.0 |
| 0.99806 | 1.3 | *1.03* | 1.03 | 0.99176 | 5.8 | *4.64* | 4.60 | 0.98625 | 10.3 | *8.29* | 8.1 |
| 0.99791 | 1.4 | *1.11* | 1.11 | 0.99163 | 5.9 | *4.72* | 4.68 | 0.98614 | 10.4 | *8.37* | 8.2 |
| 0.99776 | 1.5 | *1.19* | 1.19 | 0.99150 | 6.0 | *4.80* | 4.76 | 0.98602 | 10.5 | *8.45* | 8.3 |
| 0.99761 | 1.6 | *1.27* | 1.27 | 0.99137 | 6.1 | *4.88* | 4.84 | 0.98590 | 10.6 | *8.54* | 8.4 |
| 0.99747 | 1.7 | *1.35* | 1.35 | 0.99124 | 6.2 | *4.97* | 4.92 | 0.98579 | 10.7 | *8.62* | 8.4 |
| 0.99732 | 1.8 | *1.43* | 1.43 | 0.99111 | 6.3 | *5.05* | 5.00 | 0.98567 | 10.8 | *8.70* | 8.5 |
| 0.99717 | 1.9 | *1.51* | 1.51 | 0.99098 | 6.4 | *5.13* | 5.08 | 0.98556 | 10.9 | *8.78* | 8.6 |
| 0.99703 | 2.0 | *1.59* | 1.59 | 0.99085 | 6.5 | *5.21* | 5.16 | 0.98544 | 11.0 | *8.86* | 8.7 |
| 0.99688 | 2.1 | *1.67* | 1.67 | 0.99073 | 6.6 | *5.29* | 5.23 | 0.98532 | 11.1 | *8.94* | 8.8 |
| 0.99674 | 2.2 | *1.75* | 1.74 | 0.99060 | 6.7 | *5.37* | 5.31 | 0.98521 | 11.2 | *9.03* | 8.8 |
| 0.99659 | 2.3 | *1.83* | 1.82 | 0.99047 | 6.8 | *5.45* | 5.39 | 0.98509 | 11.3 | *9.11* | 8.9 |
| 0.99645 | 2.4 | *1.91* | 1.90 | 0.99035 | 6.9 | *5.53* | 5.47 | 0.98498 | 11.4 | *9.19* | 9.0 |
| 0.99630 | 2.5 | *1.99* | 1.98 | 0.99022 | 7.0 | *5.61* | 5.55 | 0.98487 | 11.5 | *9.27* | 9.1 |
| 0.99616 | 2.6 | *2.07* | 2.06 | 0.99009 | 7.1 | *5.69* | 5.63 | 0.98475 | 11.6 | *9.35* | 9.2 |
| 0.99602 | 2.7 | *2.15* | 2.14 | 0.98997 | 7.2 | *5.77* | 5.71 | 0.98464 | 11.7 | *9.43* | 9.2 |
| 0.99587 | 2.8 | *2.23* | 2.22 | 0.98984 | 7.3 | *5.85* | 5.79 | 0.98452 | 11.8 | *9.52* | 9.3 |
| 0.99573 | 2.9 | *2.31* | 2.30 | 0.98972 | 7.4 | *5.94* | 5.87 | 0.98441 | 11.9 | *9.60* | 9.4 |
| 0.99559 | 3.0 | *2.39* | 2.38 | 0.98960 | 7.5 | *6.02* | 5.95 | 0.98430 | 12.0 | *9.68* | 9.5 |
| 0.99545 | 3.1 | *2.47* | 2.46 | 0.98947 | 7.6 | *6.10* | 6.03 | 0.98419 | 12.1 | *9.76* | 9.6 |
| 0.99531 | 3.2 | *2.55* | 2.54 | 0.98935 | 7.7 | *6.18* | 6.11 | 0.98408 | 12.2 | *9.84* | 9.6 |
| 0.99516 | 3.3 | *2.63* | 2.62 | 0.98923 | 7.8 | *6.26* | 6.19 | 0.98396 | 12.3 | *9.92* | 9.7 |
| 0.99502 | 3.4 | *2.71* | 2.70 | 0.98911 | 7.9 | *6.34* | 6.27 | 0.98385 | 12.4 | *10.01* | 9.8 |
| 0.99488 | 3.5 | *2.79* | 2.78 | 0.98899 | 8.0 | *6.42* | 6.35 | 0.98374 | 12.5 | *10.09* | 9.9 |
| 0.99474 | 3.6 | *2.87* | 2.86 | 0.98887 | 8.1 | *6.50* | 6.42 | 0.98363 | 12.6 | *10.17* | 9.9 |
| 0.99460 | 3.7 | *2.95* | 2.93 | 0.98875 | 8.2 | *6.58* | 6.50 | 0.98352 | 12.7 | *10.25* | 10.0 |
| 0.99446 | 3.8 | *3.03* | 3.01 | 0.98863 | 8.3 | *6.67* | 6.58 | 0.98341 | 12.8 | *10.33* | 10.1 |
| 0.99432 | 3.9 | *3.11* | 3.09 | 0.98851 | 8.4 | *6.75* | 6.66 | 0.98330 | 12.9 | *10.42* | 10.2 |
| 0.99419 | 4.0 | *3.19* | 3.17 | 0.98838 | 8.5 | *6.83* | 6.74 | 0.98319 | 13.0 | *10.50* | 10.3 |
| 0.99405 | 4.1 | *3.27* | 3.25 | 0.98826 | 8.6 | *6.91* | 6.82 | 0.98308 | 13.1 | *10.58* | 10.3 |
| 0.99391 | 4.2 | *3.35* | 3.33 | 0.98814 | 8.7 | *6.99* | 6.90 | 0.98297 | 13.2 | *10.66* | 10.4 |
| 0.99378 | 4.3 | *3.44* | 3.41 | 0.98803 | 8.8 | *7.07* | 6.98 | 0.98286 | 13.3 | *10.74* | 10.5 |
| 0.99364 | 4.4 | *3.52* | 3.49 | 0.98791 | 8.9 | *7.15* | 7.06 | 0.98275 | 13.4 | *10.82* | 10.6 |

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[1] Compiled at the U. S. Bur. of Standards. This table is based upon true specific gravity instead of apparent. For concentrations of 25 alcohol by volume the apparent specific gravity is 0.97080, the true specific gravity, 0.97084. For lower concentrations the difference betw the two specific gravities is less; for higher concentrations the difference is greater.

### *Alcohol table*—Continued.

| GR. /15.56° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC | SP. GR. 15.56/15.56° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC | SP GR. 15.56/15.56° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 8264 | **13.5** | *10.91* | 10.71 | 0.97844 | **17.5** | *14.20* | 13.88 | 0.97446 | **21.5** | *17.52* | 17.05 |
| 8254 | **13.6** | *10.99* | 10.79 | 0.97834 | **17.6** | *14.28* | 13.96 | 0.97436 | **21.6** | *17.60* | 17.13 |
| 8243 | **13.7** | *11.07* | 10.87 | 0.97824 | **17.7** | *14.36* | 14.04 | 0.97425 | **21.7** | *17.68* | 17.21 |
| 8232 | **13.8** | *11.15* | 10.95 | 0.97814 | **17.8** | *14.45* | 14.12 | 0.97415 | **21.8** | *17.77* | 17.29 |
| 8221 | **13.9** | *11.23* | 11.02 | 0.97804 | **17.9** | *14.53* | 14.20 | 0.97405 | **21.9** | *17.85* | 17.37 |
| 8210 | **14.0** | *11.32* | 11.10 | 0.97794 | **18.0** | *14.61* | 14.28 | 0.97395 | **22.0** | *17.93* | 17.45 |
| 8200 | **14.1** | *11.40* | 11.18 | 0.97784 | **18.1** | *14.70* | 14.36 | 0.97385 | **22.1** | *18.02* | 17.53 |
| 8190 | **14.2** | *11.48* | 11.26 | 0.97774 | **18.2** | *14.78* | 14.43 | 0.97375 | **22.2** | *18.10* | 17.61 |
| 8179 | **14.3** | *11.56* | 11.34 | 0.97764 | **18.3** | *14.86* | 14.51 | 0.97365 | **22.3** | *18.18* | 17.69 |
| 8168 | **14.4** | *11.65* | 11.42 | 0.97754 | **18.4** | *14.94* | 14.59 | 0.97354 | **22.4** | *18.27* | 17.77 |
| 8157 | **14.5** | *11.73* | 11.50 | 0.97744 | **18.5** | *15.03* | 14.67 | 0.97344 | **22.5** | *18.35* | 17.85 |
| 8147 | **14.6** | *11.81* | 11.58 | 0.97734 | **18.6** | *15.11* | 14.75 | 0.97334 | **22.6** | *18.43* | 17.92 |
| 8136 | **14.7** | *11.89* | 11.66 | 0.97724 | **18.7** | *15.19* | 14.83 | 0.97324 | **22.7** | *18.52* | 18.00 |
| 8125 | **14.8** | *11.97* | 11.74 | 0.97714 | **18.8** | *15.27* | 14.91 | 0.97313 | **22.8** | *18.60* | 18.08 |
| 8115 | **14.9** | *12.06* | 11.82 | 0.97704 | **18.9** | *15.36* | 14.99 | 0.97303 | **22.9** | *18.68* | 18.16 |
| 8104 | **15.0** | *12.14* | 11.90 | 0.97694 | **19.0** | *15.44* | 15.07 | 0.97293 | **23.0** | *18.77* | 18.24 |
| 8093 | **15.1** | *12.22* | 11.98 | 0.97684 | **19.1** | *15.52* | 15.15 | 0.97283 | **23.1** | *18.85* | 18.32 |
| 8083 | **15.2** | *12.30* | 12.06 | 0.97674 | **19.2** | *15.61* | 15.23 | 0.97272 | **23.2** | *18.93* | 18.40 |
| 8072 | **15.3** | *12.39* | 12.13 | 0.97664 | **19.3** | *15.69* | 15.31 | 0.97262 | **23.3** | *19.02* | 18.48 |
| 8062 | **15.4** | *12.47* | 12.21 | 0.97654 | **19.4** | *15.77* | 15.39 | 0.97252 | **23.4** | *19.10* | 18.56 |
| 8051 | **15.5** | *12.55* | 12.29 | 0.97645 | **19.5** | *15.85* | 15.47 | 0.97241 | **23.5** | *19.19* | 18.64 |
| 8040 | **15.6** | *12.63* | 12.37 | 0.97635 | **19.6** | *15.94* | 15.55 | 0.97231 | **23.6** | *19.27* | 18.72 |
| 8030 | **15.7** | *12.71* | 12.45 | 0.97625 | **19.7** | *16.02* | 15.62 | 0.97221 | **23.7** | *19.35* | 18.80 |
| 8019 | **15.8** | *12.80* | 12.53 | 0.97615 | **19.8** | *16.10* | 15.70 | 0.97210 | **23.8** | *19.44* | 18.88 |
| 8009 | **15.9** | *12.88* | 12.61 | 0.97605 | **19.9** | *16.19* | 15.78 | 0.97200 | **23.9** | *19.52* | 18.96 |
| 7998 | **16.0** | *12.96* | 12.69 | 0.97596 | **20.0** | *16.27* | 15.86 | 0.97189 | **24.0** | *19.60* | 19.04 |
| 7988 | **16.1** | *13.04* | 12.77 | 0.97586 | **20.1** | *16.35* | 15.94 | 0.97179 | **24.1** | *19.69* | 19.11 |
| 7977 | **16.2** | *13.13* | 12.85 | 0.97576 | **20.2** | *16.44* | 16.02 | 0.97168 | **24.2** | *19.77* | 19.19 |
| 7967 | **16.3** | *13.21* | 12.93 | 0.97566 | **20.3** | *16.52* | 16.10 | 0.97158 | **24.3** | *19.86* | 19.27 |
| 7956 | **16.4** | *13.29* | 13.01 | 0.97556 | **20.4** | *16.60* | 16.18 | 0.97147 | **24.4** | *19.94* | 19.35 |
| 7946 | **16.5** | *13.37* | 13.09 | 0.97546 | **20.5** | *16.68* | 16.26 | 0.97137 | **24.5** | *20.02* | 19.43 |
| 7936 | **16.6** | *13.46* | 13.17 | 0.97536 | **20.6** | *16.77* | 16.34 | 0.97126 | **24.6** | *20.11* | 19.51 |
| 7925 | **16.7** | *13.54* | 13.25 | 0.97526 | **20.7** | *16.85* | 16.42 | 0.97116 | **24.7** | *20.19* | 19.59 |
| 7915 | **16.8** | *13.62* | 13.32 | 0.97516 | **20.8** | *16.93* | 16.50 | 0.97105 | **24.8** | *20.28* | 19.67 |
| 7905 | **16.9** | *13.70* | 13.40 | 0.97506 | **20.9** | *17.02* | 16.58 | 0.97095 | **24.9** | *20.36* | 19.75 |
| 7895 | **17.0** | *13.79* | 13.48 | 0.97496 | **21.0** | *17.10* | 16.66 | 0.97084 | **25.0** | *20.44* | 19.83 |
| 7885 | **17.1** | *13.87* | 13.56 | 0.97486 | **21.1** | *17.18* | 16.74 | | | | |
| 7875 | **17.2** | *13.95* | 13.64 | 0.97476 | **21.2** | *17.27* | 16.81 | | | | |
| 7864 | **17.3** | *14.03* | 13.72 | 0.97466 | **21.3** | *17.35* | 16.89 | | | | |
| 7854 | **17.4** | *14.12* | 13.80 | 0.97456 | **21.4** | *17.43* | 16.97 | | | | |

20 *Relation between specific gravity at 20/20°C. and alcohol content of mixtures of ethyl alcohol and water.*[1]

| SP GR. 20/20° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 cc | SP GR. 20/20° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 cc | SP GR. 20/20° | PER CENT BY VOL. | PER CENT BY WT. | G PFR 100 c |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.00000 | **0.0** | *0.00* | 0.00 | 0.99349 | **4.5** | *3.58* | 3.55 | 0.98770 | **9.0** | *7.20* | 7.1 |
| 0.99985 | **0.1** | *0.08* | 0.08 | 0.99336 | **4.6** | *3.66* | 3.63 | 0.98758 | **9.1** | *7.29* | 7.1 |
| 0.99970 | **0.2** | *0.16* | 0.16 | 0.99322 | **4.7** | *3.74* | 3.71 | 0.98746 | **9.2** | *7.37* | 7.2 |
| 0.99955 | **0.3** | *0.24* | 0.24 | 0.99308 | **4.8** | *3.82* | 3.79 | 0.98734 | **9.3** | *7.45* | 7.3 |
| 0.99939 | **0.4** | *0.32* | 0.32 | 0.99295 | **4.9** | *3.90* | 3.87 | 0.98722 | **9.4** | *7.53* | 7.4 |
| 0.99924 | **0.5** | *0.40* | 0.40 | 0.99281 | **5.0** | *3.98* | 3.95 | 0.98710 | **9.5** | *7.61* | 7.5 |
| 0.99910 | **0.6** | *0.47* | 0.47 | 0.99268 | **5.1** | *4.06* | 4.03 | 0.98698 | **9.6** | *7.69* | 7.5 |
| 0.99895 | **0.7** | *0.55* | 0.55 | 0.99255 | **5.2** | *4.14* | 4.10 | 0.98686 | **9.7** | *7.77* | 7.6 |
| 0.99880 | **0.8** | *0.63* | 0.63 | 0.99241 | **5.3** | *4.22* | 4.18 | 0.98674 | **9.8** | *7.85* | 7.7 |
| 0.99866 | **0.9** | *0.71* | 0.71 | 0.99228 | **5.4** | *4.30* | 4.26 | 0.98662 | **9.9** | *7.93* | 7.8 |
| 0.99851 | **1.0** | *0.79* | 0.79 | 0.99215 | **5.5** | *4.38* | 4.34 | 0.98650 | **10.0** | *8.02* | 7.8 |
| 0.99836 | **1.1** | *0.87* | 0.87 | 0.99201 | **5.6** | *4.46* | 4.42 | 0.98637 | **10.1** | *8.10* | 7.9 |
| 0.99821 | **1.2** | *0.95* | 0.95 | 0.99188 | **5.7** | *4.54* | 4.50 | 0.98626 | **10.2** | *8.18* | 8.0 |
| 0.99807 | **1.3** | *1.03* | 1.03 | 0.99174 | **5.8** | *4.62* | 4.58 | 0.98614 | **10.3** | *8.26* | 8.1 |
| 0.99792 | **1.4** | *1.11* | 1.11 | 0.99161 | **5.9** | *4.70* | 4.66 | 0.98602 | **10.4** | *8.34* | 8.2 |
| 0.99777 | **1.5** | *1.19* | 1.19 | 0.99148 | **6.0** | *4.78* | 4.74 | 0.98590 | **10.5** | *8.42* | 8.2 |
| 0.99763 | **1.6** | *1.27* | 1.26 | 0.99135 | **6.1** | *4.87* | 4.82 | 0.98578 | **10.6** | *8.50* | 8.3 |
| 0.99748 | **1.7** | *1.35* | 1.34 | 0.99122 | **6.2** | *4.95* | 4.89 | 0.98566 | **10.7** | *8.58* | 8.4 |
| 0.99733 | **1.8** | *1.43* | 1.42 | 0.99109 | **6.3** | *5.03* | 4.97 | 0.98554 | **10.8** | *8.66* | 8.5 |
| 0.99719 | **1.9** | *1.51* | 1.50 | 0.99096 | **6.4** | *5.11* | 5.05 | 0.98542 | **10.9** | *8.75* | 8.6 |
| 0.99704 | **2.0** | *1.59* | 1.58 | 0.99083 | **6.5** | *5.19* | 5.13 | 0.98530 | **11.0** | *8.83* | 8.6 |
| 0.99689 | **2.1** | *1.67* | 1.66 | 0.99070 | **6.6** | *5.27* | 5.21 | 0.98518 | **11.1** | *8.91* | 8.7 |
| 0.99675 | **2.2** | *1.75* | 1.74 | 0.99057 | **6.7** | *5.35* | 5.29 | 0.98506 | **11.2** | *8.99* | 8.8 |
| 0.99661 | **2.3** | *1.82* | 1.81 | 0.99045 | **6.8** | *5.43* | 5.37 | 0.98494 | **11.3** | *9.07* | 8.9 |
| 0.99646 | **2.4** | *1.90* | 1.89 | 0.99032 | **6.9** | *5.51* | 5.45 | 0.98482 | **11.4** | *9.15* | 9.0 |
| 0.99632 | **2.5** | *1.98* | 1.97 | 0.99020 | **7.0** | *5.59* | 5.53 | 0.98470 | **11.5** | *9.23* | 9.0 |
| 0.99618 | **2.6** | *2.06* | 2.05 | 0.99007 | **7.1** | *5.67* | 5.60 | 0.98459 | **11.6** | *9.32* | 9.1 |
| 0.99603 | **2.7** | *2.14* | 2.13 | 0.98994 | **7.2** | *5.75* | 5.68 | 0.98447 | **11.7** | *9.40* | 9.2 |
| 0.99589 | **2.8** | *2.22* | 2.21 | 0.98981 | **7.3** | *5.83* | 5.76 | 0.98435 | **11.8** | *9.48* | 9.3 |
| 0.99574 | **2.9** | *2.30* | 2.29 | 0.98969 | **7.4** | *5.91* | 5.84 | 0.98424 | **11.9** | *9.56* | 9.3 |
| 0.99560 | **3.0** | *2.38* | 2.37 | 0.98956 | **7.5** | *5.99* | 5.92 | 0.98412 | **12.0** | *9.64* | 9.4 |
| 0.99546 | **3.1** | *2.46* | 2.45 | 0.98944 | **7.6** | *6.07* | 6.00 | 0.98400 | **12.1** | *9.72* | 9.5 |
| 0.99531 | **3.2** | *2.54* | *2.53* | 0.98931 | **7.7** | *6.15* | 6.08 | 0.98388 | **12.2** | *9.80* | 9.6 |
| 0.99517 | **3.3** | *2.62* | 2.60 | 0.98919 | **7.8** | *6.24* | 6.16 | 0.98377 | **12.3** | *9.89* | 9.7 |
| 0.99503 | **3.4** | *2.70* | 2.68 | 0.98906 | **7.9** | *6.32* | 6.24 | 0.98365 | **12.4** | *9.97* | 9.7 |
| 0.99489 | **3.5** | *4.78* | 2.76 | 0.98893 | **8.0** | *6.40* | 6.32 | 0.98354 | **12.5** | *10.05* | 9.8 |
| 0.99475 | **3.6** | *4.86* | 2.84 | 0.98881 | **8.1** | *6.48* | 6.39 | 0.98342 | **12.6** | *10.13* | 9.9 |
| 0.99461 | **3.7** | *4.94* | 2.92 | 0.98869 | **8.2** | *6.56* | 6.47 | 0.98330 | **12.7** | *10.21* | 10.0 |
| 0.99447 | **3.8** | *3.02* | 3.00 | 0.98857 | **8.3** | *6.64* | 6.55 | 0.98318 | **12.8** | *10.29* | 10.1 |
| 0.99433 | **3.9** | *3.10* | 3.08 | 0.98845 | **8.4** | *6.72* | 6.63 | 0.98307 | **12.9** | *10.38* | 10.1 |
| 0.99419 | **4.0** | *3.18* | 3.16 | 0.98833 | **8.5** | *6.80* | 6.71 | 0.98296 | **13.0** | *10.46* | 10.2 |
| 0.99405 | **4.1** | *3.26* | 3.24 | 0.98820 | **8.6** | *6.88* | 6.79 | 0.98285 | **13.1** | *10.54* | 10.3 |
| 0.99391 | **4.2** | *3.34* | 3.32 | 0.98807 | **8.7** | *6.96* | 6.87 | 0.98274 | **13.2** | *10.62* | 10.4 |
| 0.99377 | **4.3** | *3.42* | 3.39 | 0.98794 | **8.8** | *7.04* | 6.95 | 0.98263 | **13.3** | *10.70* | 10.5 |
| 0.99363 | **4.4** | *3.50* | 3.47 | 0.98782 | **8.9** | *7.12* | 7.03 | 0.98251 | **13.4** | *10.78* | 10.5 |

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[1] Compiled at the U. S. Bureau of Standards. This table is based upon true specific gravity instead of apparent. In a solution containing 2 alcohol by volume, the apparent specific gravity is 0.96980, the true specific gravity 0.96983. For lower concentrations the difference betw the two specific gravities is less; for higher concentrations the difference is greater.

## *Alcohol table.*—Continued. 20

| GR. /20° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC | SP GR. 20/20° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC | SP GR. 20/20° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 8239 | **13.5** | *10.86* | 10.66 | 0.97797 | **17.5** | *14.15* | 13.81 | 0.97371 | **21.5** | *17.46* | 16.97 |
| 8227 | **13.6** | *10.95* | 10.74 | 0.97786 | **17.6** | *14.23* | 13.89 | 0.97360 | **21.6** | *17.54* | 17.05 |
| 8216 | **13.7** | *11.03* | 10.81 | 0.97775 | **17.7** | *14.31* | 13.97 | 0.97350 | **21.7** | *17.63* | 17.13 |
| 8204 | **13.8** | *11.11* | 10.89 | 0.97764 | **17.8** | *14.40* | 14.05 | 0.97339 | **21.8** | *17.71* | 17.21 |
| 8193 | **13.9** | *11.19* | 10.97 | 0.97754 | **17.9** | *14.48* | 14.13 | 0.97328 | **21.9** | *17.79* | 17.29 |
| 8182 | **14.0** | *11.28* | 11.05 | 0.97743 | **18.0** | *14.56* | 14.21 | 0.97317 | **22.0** | *17.88* | 17.37 |
| 8171 | **14.1** | *11.36* | 11.13 | 0.97732 | **18.1** | *14.64* | 14.29 | 0.97306 | **22.1** | *17.96* | 17.45 |
| 8159 | **14.2** | *11.44* | 11.21 | 0.97721 | **18.2** | *14.73* | 14.37 | 0.97295 | **22.2** | *18.04* | 17.52 |
| 8148 | **14.3** | *11.52* | 11.29 | 0.97711 | **18.3** | *14.81* | 14.45 | 0.97285 | **22.3** | *18.13* | 17.60 |
| 8137 | **14.4** | *11.60* | 11.37 | 0.97700 | **18.4** | *14.89* | 14.52 | 0.97274 | **22.4** | *18.21* | 17.68 |
| 8126 | **14.5** | *11.68* | 11.44 | 0.97690 | **18.5** | *14.97* | 14.60 | 0.97263 | **22.5** | *18.29* | 17.76 |
| 8115 | **14.6** | *11.77* | 11.52 | 0.97679 | **18.6** | *15.06* | 14.68 | 0.97252 | **22.6** | *18.38* | 17.84 |
| 8103 | **14.7** | *11.85* | 11.60 | 0.97668 | **18.7** | *15.14* | 14.76 | 0.97241 | **22.7** | *18.46* | 17.92 |
| 8092 | **14.8** | *11.93* | 11.68 | 0.97657 | **18.8** | *15.22* | 14.84 | 0.97230 | **22.8** | *18.54* | 18.00 |
| 8081 | **14.9** | *12.01* | 11.76 | 0.97646 | **18.9** | *15.30* | 14.92 | 0.97219 | **22.9** | *18.63* | 18.08 |
| 8070 | **15.0** | *12.09* | 11.84 | 0.97636 | **19.0** | *15.39* | 15.00 | 0.97208 | **23.0** | *18.71* | 18.16 |
| 8058 | **15.1** | *12.18* | 11.92 | 0.97626 | **19.1** | *15.47* | 15.08 | 0.97197 | **23.1** | *18.79* | 18.24 |
| 8047 | **15.2** | *12.26* | 12.00 | 0.97616 | **19.2** | *15.55* | 15.16 | 0.97185 | **23.2** | *18.88* | 18.31 |
| 8036 | **15.3** | *12.34* | 12.08 | 0.97605 | **19.3** | *15.64* | 15.23 | 0.97174 | **23.3** | *18.96* | 18.39 |
| 8025 | **15.4** | *12.42* | 12.16 | 0.97595 | **19.4** | *15.72* | 15.31 | 0.97163 | **23.4** | *19.04* | 18.47 |
| 8014 | **15.5** | *12.50* | 12.23 | 0.97584 | **19.5** | *15.80* | 15.39 | 0.97152 | **23.5** | *19.13* | 18.55 |
| 8003 | **15.6** | *12.59* | 12.31 | 0.97574 | **19.6** | *15.88* | 15.47 | 0.97141 | **23.6** | *19.21* | 18.63 |
| 7992 | **15.7** | *12.67* | 12.39 | 0.97563 | **19.7** | *15.97* | 15.55 | 0.97130 | **23.7** | *19.29* | 18.71 |
| 7981 | **15.8** | *12.75* | 12.47 | 0.97553 | **19.8** | *16.05* | 15.63 | 0.97118 | **23.8** | *19.38* | 18.79 |
| 7970 | **15.9** | *12.83* | 12.55 | 0.97542 | **19.9** | *16.13* | 15.71 | 0.97107 | **23.9** | *19.46* | 18.87 |
| 7959 | **16.0** | *12.92* | 12.63 | 0.97531 | **20.0** | *16.21* | 15.79 | 0.97096 | **24.0** | *19.55* | 18.94 |
| 7948 | **16.1** | *13.00* | 12.71 | 0.97521 | **20.1** | *16.30* | 15.87 | 0.97084 | **24.1** | *19.63* | 19.02 |
| 7937 | **16.2** | *13.08* | 12.79 | 0.97511 | **20.2** | *16.38* | 15.95 | 0.97073 | **24.2** | *19.71* | 19.10 |
| 7926 | **16.3** | *13.16* | 12.87 | 0.97500 | **20.3** | *16.46* | 16.02 | 0.97062 | **24.3** | *19.80* | 19.18 |
| 7915 | **16.4** | *13.24* | 12.95 | 0.97489 | **20.4** | *16.55* | 16.10 | 0.97051 | **24.4** | *19.88* | 19.26 |
| 7905 | **16.5** | *13.33* | 13.02 | 0.97478 | **20.5** | *16.63* | 16.18 | 0.97040 | **24.5** | *19.96* | 19.34 |
| 7894 | **16.6** | *13.41* | 13.10 | 0.97467 | **20.6** | *16.71* | 16.26 | 0.97028 | **24.6** | *20.05* | 19.42 |
| 7883 | **16.7** | *13.49* | 13.18 | 0.97456 | **20.7** | *16.80* | 16.34 | 0.97017 | **24.7** | *20.13* | 19.50 |
| 7872 | **16.8** | *13.57* | 13.26 | 0.97445 | **20.8** | *16.88* | 16.42 | 0.97006 | **24.8** | *20.22* | 19.58 |
| 7862 | **16.9** | *13.66* | 13.34 | 0.97435 | **20.9** | *16.96* | 16.50 | 0.96994 | **24.9** | *20.30* | 19.66 |
| 7851 | **17.0** | *13.74* | 13.42 | 0.97424 | **21.0** | *17.04* | 16.58 | 0.96983 | 25.0 | *20.38* | 19.73 |
| 7840 | **17.1** | *13.82* | 13.50 | 0.97414 | **21.1** | *17.13* | 16.66 |  |  |  |  |
| 7829 | **17.2** | *13.90* | 13.58 | 0.97404 | **21.2** | *17.21* | 16.73 |  |  |  |  |
| 7818 | **17.3** | *13.98* | 13.66 | 0.97393 | **21.3** | *17.29* | 16.81 |  |  |  |  |
| 7807 | **17.4** | *14.07* | 13.74 | 0.97382 | **21.4** | *17.38* | 16.89 |  |  |  |  |

21 *Relation between specific gravity at 25/25°C. and alcohol content of mixtures of ethyl alcohol and water.*[1]

| SP GR. 25/25° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC | SP. GR. 25/25° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC | SP. GR. 25/25° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.00000 | 0.0 | *0.00* | 0.00 | 0.99348 | **4.5** | *3.57* | 3.53 | 0.98762 | **9.0** | *7.18* | 7.0 |
| 0.99985 | 0.1 | *0.08* | 0.08 | 0.99335 | **4.6** | *3.65* | 3.61 | 0.98749 | **9.1** | *7.26* | 7.1 |
| 0.99970 | 0.2 | *0.16* | 0.16 | 0.99321 | **4.7** | *3.73* | 3.69 | 0.98737 | **9.2** | *7.34* | 7.2 |
| 0.99955 | 0.3 | *0.24* | 0.24 | 0.99307 | **4.8** | *3.81* | 3.77 | 0.98724 | **9.3** | *7.42* | 7.3 |
| 0.99940 | 0.4 | *0.31* | 0.31 | 0.99293 | **4.9** | *3.89* | 3.85 | 0.98712 | **9.4** | *7.50* | 7.3 |
| 0.99925 | 0.5 | *0.39* | 0.39 | 0.99280 | **5.0** | *3.97* | 3.93 | 0.98699 | **9.5** | *7.58* | 7.4 |
| 0.99910 | 0.6 | *0.47* | 0.47 | 0.99266 | **5.1** | *4.05* | 4.00 | 0.98686 | **9.6** | *7.66* | 7.5 |
| 0.99895 | 0.7 | *0.55* | 0.55 | 0.99253 | **5.2** | *4.13* | 4.08 | 0.98674 | **9.7** | *7.74* | 7.6 |
| 0.99881 | 0.8 | *0.63* | 0.63 | 0.99239 | **5.3** | *4.20* | 4.16 | 0.98661 | **9.8** | *7.82* | 7.6 |
| 0.99866 | 0.9 | *0.71* | 0.71 | 0.99226 | **5.4** | *4.28* | 4.24 | 0.98649 | **9.9** | *7.90* | 7.7 |
| 0.99851 | 1.0 | *0.79* | 0.79 | 0.99212 | **5.5** | *4.36* | 4.32 | 0.98637 | **10.0** | *7.98* | 7.8 |
| 0.99836 | 1.1 | *0.87* | 0.86 | 0.99199 | **5.6** | *4.44* | 4.40 | 0.98624 | **10.1** | *8.06* | 7.9 |
| 0.99822 | 1.2 | *0.95* | 0.94 | 0.99185 | **5.7** | *4.52* | 4.47 | 0.98612 | **10.2** | *8.14* | 8.0 |
| 0.99807 | 1.3 | *1.03* | 1.02 | 0.99172 | **5.8** | *4.60* | 4.55 | 0.98600 | **10.3** | *8.22* | 8.0 |
| 0.99792 | 1.4 | *1.10* | 1.10 | 0.99159 | **5.9** | *4.68* | 4.63 | 0.98587 | **10.4** | *8.31* | 8.1 |
| 0.99777 | 1.5 | *1.18* | 1.18 | 0.99145 | **6.0** | *4.76* | 4.71 | 0.98575 | **10.5** | *8.39* | 8.2 |
| 0.99763 | 1.6 | *1.26* | 1.26 | 0.99132 | **6.1** | *4.84* | 4.79 | 0.98563 | **10.6** | *8.47* | 8.3 |
| 0.99748 | 1.7 | *1.34* | 1.33 | 0.99119 | **6.2** | *4.93* | 4.87 | 0.98550 | **10.7** | *8.55* | 8.4 |
| 0.99733 | 1.8 | *1.42* | 1.41 | 0.99106 | **6.3** | *5.01* | 4.95 | 0.98538 | **10.8** | *8.63* | 8.4 |
| 0.99718 | 1.9 | *1.50* | 1.49 | 0.99093 | **6.4** | *5.09* | 5.02 | 0.98525 | **10.9** | *8.71* | 8.5 |
| 0.99704 | 1.0 | *1.58* | 1.57 | 0.99080 | **6.5** | *5.17* | 5.10 | 0.98513 | **11.0** | *8.79* | 8.6 |
| 0.99689 | 2.1 | *1.66* | 1.65 | 0.99067 | **6.6** | *6.25* | 5.18 | 0.98501 | **11.1** | *8.87* | 8.7 |
| 0.99675 | 2.2 | *1.74* | 1.73 | 0.99054 | **6.7** | *5.33* | 5.26 | 0.98488 | **11.2** | *8.95* | 8.7 |
| 0.99660 | 2.3 | *1.82* | 1.81 | 0.99041 | **6.8** | *5.41* | 5.34 | 0.98476 | **11.3** | *9.03* | 8.8 |
| 0.99646 | 2.4 | *1.90* | 1.88 | 0.99027 | **6.9** | *5.49* | 5.42 | 0.98464 | **11.4** | *9.12* | 8.9 |
| 0.99631 | 2.5 | *1.98* | 1.96 | 0.99014 | **7.0** | *5.57* | 5.50 | 0.98451 | **11.5** | *9.20* | 9.0 |
| 0.99617 | 2.6 | *2.06* | 2.04 | 0.99001 | **7.1** | *5.65* | 5.57 | 0.98439 | **11.6** | *9.28* | 9.1 |
| 0.99603 | 2.7 | *2.13* | 2.12 | 0.98988 | **7.2** | *5.73* | 5.65 | 0.98427 | **11.7** | *9.36* | 9.1 |
| 0.99589 | 2.8 | *2.21* | 2.20 | 0.98975 | **7.3** | *5.81* | 5.73 | 0.98415 | **11.8** | *9.44* | 9.2 |
| 0.99574 | 2.9 | *2.29* | 2.28 | 0.98962 | **7.4** | *5.89* | 5.81 | 0.98402 | **11.9** | *9.52* | 9.3 |
| 0.99560 | 3.0 | *2.37* | 2.36 | 0.98950 | **7.5** | *5.97* | 5.89 | 0.98390 | **12.0** | *9.60* | 9.4 |
| 0.99546 | 3.1 | *2.45* | 2.43 | 0.98937 | **7.6** | *6.05* | 5.97 | 0.98378 | **12.1** | *9.68* | 9.5 |
| 0.99531 | 3.2 | *2.53* | 2.51 | 0.98925 | **7.7** | *6.13* | 6.04 | 0.98366 | **12.2** | *9.77* | 9.5 |
| 0.99517 | 3.3 | *2.61* | 2.59 | 0.98912 | **7.8** | *6.21* | 6.12 | 0.98354 | **12.3** | *9.85* | 9.6 |
| 0.99503 | 3.4 | *2.69* | 2.67 | 0.98899 | **7.9** | *6.29* | 6.20 | 0.98341 | **12.4** | *9.93* | 9.7 |
| 0.99488 | 3.5 | *2.77* | 2.75 | 0.98887 | **8.0** | *6.37* | 6.28 | 0.98329 | **12.5** | *10.01* | 9.8 |
| 0.99474 | 3.6 | *2.85* | 2.83 | 0.98874 | **8.1** | *6.45* | 6.36 | 0.98317 | **12.6** | *10.09* | 9.8 |
| 0.99460 | 3.7 | *2.93* | 2.90 | 0.98861 | **8.2** | *6.53* | 6.44 | 0.98305 | **12.7** | *10.17* | 9.9 |
| 0.99446 | 3.8 | *3.01* | 2.98 | 0.98849 | **8.3** | *6.61* | 6.52 | 0.98293 | **12.8** | *10.25* | 10.0 |
| 0.99432 | 3.9 | *3.09* | 3.06 | 0.98836 | **8.4** | *6.69* | 6.59 | 0.98281 | **12.9** | *10.33* | 10.1 |
| 0.99418 | 4.0 | *3.17* | 3.14 | 0.98824 | **8.5** | *6.77* | 6.67 | 0.98269 | **13.0** | *10.42* | 10.2 |
| 0.99404 | 4.1 | *3.25* | 3.22 | 0.98811 | **8.6** | *6.85* | 6.75 | 0.98257 | **13.1** | *10.50* | 10.2 |
| 0.99390 | 4.2 | *3.33* | 3.30 | 0.98799 | **8.7** | *6.93* | 6.83 | 0.98245 | **13.2** | *10.58* | 10.3 |
| 0.99376 | 4.3 | *3.41* | 3.38 | 0.98787 | **8.8** | *7.01* | 6.91 | 0.98233 | **13.3** | *10.66* | 10.4 |
| 0.99362 | 4.4 | *3.49* | 3.45 | 0.98774 | **8.9** | *7.09* | 6.99 | 0.98221 | **13.4** | *10.74* | 10.5 |

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[1] Compiled at the U. S. Bureau of Standards. This table is based upon true specific gravity instead of apparent. For concentration of 25 by volume the apparent specific gravity is 0.96872, the true specific gravity 0.96876. For lower concentration the difference between the t specific gravities is less; for higher concentration it is relatively greater.

## Alcohol table.—Continued.

| SP GR. 25/25° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC | SP. GR. 25/25° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC | SP. GR. 25/25° | PER CENT BY VOL. | PER CENT BY WT. | G PER 100 CC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 98209 | 13.5 | 10.82 | 10.60 | 0.97744 | 17.5 | 14.10 | 13.74 | 0.97291 | 21.5 | 17.40 | 16.88 |
| 98197 | 13.6 | 10.90 | 10.68 | 0.97733 | 17.6 | 14.18 | 13.82 | 0.97280 | 21.6 | 17.48 | 16.96 |
| 98185 | 13.7 | 10.99 | 10.76 | 0.97722 | 17.7 | 14.26 | 13.90 | 0.97268 | 21.7 | 17.57 | 17.04 |
| 98174 | 13.8 | 11.07 | 10.83 | 0.97711 | 17.8 | 14.34 | 13.97 | 0.97256 | 21.8 | 17.65 | 17.11 |
| 98162 | 13.9 | 11.15 | 10.91 | 0.97699 | 17.9 | 14.43 | 14.05 | 0.97245 | 21.9 | 17.73 | 17.19 |
| 98151 | 14.0 | 11.23 | 10.99 | 0.97687 | 18.0 | 14.51 | 14.13 | 0.97233 | 22.0 | 17.81 | 17.27 |
| 98139 | 14.1 | 11.31 | 11.07 | 0.97676 | 18.1 | 14.59 | 14.21 | 0.97222 | 22.1 | 17.90 | 17.35 |
| 98127 | 14.2 | 11.39 | 11.15 | 0.97665 | 18.2 | 14.67 | 14.29 | 0.97210 | 22.2 | 17.98 | 17.43 |
| 98115 | 14.3 | 11.48 | 11.23 | 0.97653 | 18.3 | 14.75 | 14.37 | 0.97198 | 22.3 | 18.06 | 17.51 |
| 98103 | 14.4 | 11.56 | 11.30 | 0.97642 | 18.4 | 14.84 | 14.45 | 0.97187 | 22.4 | 18.15 | 17.59 |
| 98092 | 14.5 | 11.64 | 11.38 | 0.97630 | 18.5 | 14.92 | 14.52 | 0.97175 | 22.5 | 18.23 | 17.66 |
| 98080 | 14.6 | 11.72 | 11.46 | 0.97619 | 18.6 | 15.00 | 14.60 | 0.97163 | 22.6 | 18.31 | 17.74 |
| 98068 | 14.7 | 11.80 | 11.54 | 0.97608 | 18.7 | 15.08 | 14.68 | 0.97151 | 22.7 | 18.40 | 17.82 |
| 98057 | 14.8 | 11.88 | 11.62 | 0.97597 | 18.8 | 15.17 | 14.76 | 0.97140 | 22.8 | 18.48 | 17.90 |
| 98045 | 14.9 | 11.97 | 11.70 | 0.97585 | 18.9 | 15.25 | 14.84 | 0.97128 | 22.9 | 18.56 | 17.98 |
| 98033 | 15.0 | 12.05 | 11.78 | 0.97574 | 19.0 | 15.33 | 14.92 | 0.97116 | 23.0 | 18.65 | 18.06 |
| 98021 | 15.1 | 12.13 | 11.85 | 0.97563 | 19.1 | 15.41 | 14.99 | 0.97104 | 23.1 | 18.73 | 18.13 |
| 98009 | 15.2 | 12.21 | 11.93 | 0.97551 | 19.2 | 15.50 | 15.07 | 0.97092 | 23.2 | 18.81 | 18.21 |
| 97997 | 15.3 | 12.29 | 12.01 | 0.97540 | 19.3 | 15.58 | 15.15 | 0.97080 | 23.3 | 18.90 | 18.29 |
| 97986 | 15.4 | 12.37 | 12.09 | 0.97529 | 19.4 | 15.66 | 15.23 | 0.97069 | 23.4 | 18.98 | 18.37 |
| 97974 | 15.5 | 12.46 | 12.17 | 0.97518 | 19.5 | 15.74 | 15.31 | 0.97057 | 23.5 | 19.06 | 18.45 |
| 97962 | 15.6 | 12.54 | 12.25 | 0.97507 | 19.6 | 15.83 | 15.39 | 0.97045 | 23.6 | 19.15 | 18.53 |
| 97951 | 15.7 | 12.62 | 12.33 | 0.97496 | 19.7 | 15.91 | 15.47 | 0.97033 | 23.7 | 19.23 | 18.61 |
| 97939 | 15.8 | 12.70 | 12.40 | 0.97485 | 19.8 | 15.99 | 15.54 | 0.97021 | 23.8 | 19.31 | 18.68 |
| 97928 | 15.9 | 12.78 | 12.48 | 0.97473 | 19.9 | 16.07 | 15.62 | 0.97009 | 23.9 | 19.40 | 18.76 |
| 97916 | 16.0 | 12.87 | 12.56 | 0.97462 | 20.0 | 16.16 | 15.70 | 0.96997 | 24.0 | 19.48 | 18.84 |
| 97905 | 16.1 | 12.95 | 12.64 | 0.97451 | 20.1 | 16.24 | 15.78 | 0.96985 | 24.1 | 19.57 | 18.92 |
| 97893 | 16.2 | 13.03 | 12.72 | 0.97440 | 20.2 | 16.32 | 15.86 | 0.96973 | 24.2 | 19.65 | 19.00 |
| 97882 | 16.3 | 13.11 | 12.80 | 0.97428 | 20.3 | 16.41 | 15.94 | 0.96961 | 24.3 | 19.73 | 19.08 |
| 97870 | 16.4 | 13.19 | 12.87 | 0.97417 | 20.4 | 16.49 | 16.02 | 0.96949 | 24.4 | 19.82 | 19.16 |
| 97858 | 16.5 | 13.28 | 12.95 | 0.97406 | 20.5 | 16.57 | 16.09 | 0.96937 | 24.5 | 19.90 | 19.23 |
| 97847 | 16.6 | 13.36 | 13.03 | 0.97394 | 20.6 | 16.65 | 16.17 | 0.96925 | 24.6 | 19.98 | 19.31 |
| 97835 | 16.7 | 13.44 | 13.11 | 0.97383 | 20.7 | 16.74 | 16.25 | 0.96913 | 24.7 | 20.07 | 19.39 |
| 97824 | 16.8 | 13.52 | 13.19 | 0.97371 | 20.8 | 16.82 | 16.33 | 0.96901 | 24.8 | 20.15 | 19.47 |
| 97812 | 16.9 | 13.60 | 13.27 | 0.97360 | 20.9 | 16.90 | 16.41 | 0.96889 | 24.9 | 20.23 | 19.55 |
| 97801 | 17.0 | 13.69 | 13.35 | 0.97349 | 21.0 | 16.98 | 16.49 | 0.96876 | 25.0 | 20.32 | 19.63 |
| 97789 | 17.1 | 13.77 | 13.42 | 0.97337 | 21.1 | 17.07 | 16.56 | | | | |
| 97778 | 17.2 | 13.85 | 13.50 | 0.97326 | 21.2 | 17.15 | 16.64 | | | | |
| 97767 | 17.3 | 13.93 | 13.58 | 0.97314 | 21.3 | 17.23 | 16.72 | | | | |
| 97755 | 17.4 | 14.01 | 13.66 | 0.97303 | 21.4 | 17.32 | 16.80 | | | | |

22 *Alcohol table for calculating the percentages of alcohol in mixtures of ethyl alcohol and water from their Zeiss immersion refractometer readings at 17.5–25°C.*[1]

| SCALE READING | 17.5°C. | | 18° C. | | 19° C. | | 20° C. | | 21° C. |
|---|---|---|---|---|---|---|---|---|---|
| | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume |
| 13.2 | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| 13.4 | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| 13.6 | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| 13.8 | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| 14.0 | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| 14.2 | .... | .... | .... | .... | .... | .... | .... | .... | 0.04 |
| 14.4 | .... | .... | .... | .... | .... | .... | .... | .... | 0.21 |
| 14.6 | .... | .... | .... | .... | .... | .... | 0.16 | *0.13* | 0.38 |
| 14.8 | .... | .... | .... | .... | 0.14 | *0.11* | 0.34 | *0.27* | 0.55 |
| 15.0 | 0.00 | *0.00* | 0.10 | *0.08* | 0.31 | *0.24* | 0.52 | *0.41* | 0.73 |
| 15.2 | 0.17 | *0.13* | 0.27 | *0.21* | 0.48 | *0.38* | 0.69 | *0.55* | 0.91 |
| 15.4 | 0.34 | *0.27* | 0.44 | *0.35* | 0.65 | *0.51* | 0.85 | *0.68* | 1.07 |
| 15.6 | 0.51 | *0.40* | 0.60 | *0.48* | 0.82 | *0.65* | 1.03 | *0.82* | 1.24 |
| 15.8 | 0.68 | *0.54* | 0.78 | *0.62* | 0.99 | *0.79* | 1.21 | *0.96* | 1.40 |
| 16.0 | 0.84 | *0.67* | 0.94 | *0.75* | 1.17 | *0.93* | 1.36 | *1.08* | 1.55 |
| 16.2 | 1.02 | *0.81* | 1.12 | *0.89* | 1.32 | *1.05* | 1.51 | *1.20* | 1.70 |
| 16.4 | 1.18 | *0.94* | 1.29 | *1.02* | 1.47 | *1.17* | 1.66 | *1.32* | 1.85 |
| 16.6 | 1.34 | *1.06* | 1.43 | *1.13* | 1.62 | *1.29* | 1.81 | *1.44* | 2.00 |
| 16.8 | 1.49 | *1.18* | 1.57 | *1.25* | 1.77 | *1.41* | 1.96 | *1.56* | 2.15 |
| 17.0 | 1.63 | *1.30* | 1.72 | *1.37* | 1.92 | *1.53* | 2.11 | *1.68* | 2.30 |
| 17.2 | 1.77 | *1.41* | 1.87 | *1.49* | 2.06 | *1.64* | 2.26 | *1.80* | 2.45 |
| 17.4 | 1.92 | *1.53* | 2.01 | *1.60* | 2.21 | *1.76* | 2.41 | *1.92* | 2.59 |
| 17.6 | 2.07 | *1.65* | 2.16 | *1.72* | 2.36 | *1.88* | 2.56 | *2.04* | 2.74 |
| 17.8 | 2.21 | *1.76* | 2.31 | *1.84* | 2.51 | *2.00* | 2.70 | *2.15* | 2.89 |
| 18.0 | 2.36 | *1.88* | 2.45 | *1.95* | 2.66 | *2.12* | 2.85 | *2.27* | 3.04 |
| 18.2 | 2.50 | *2.00* | 2.60 | *2.07* | 2.81 | *2.24* | 3.00 | *2.39* | 3.19 |
| 18.4 | 2.65 | *2.11* | 2.75 | *2.19* | 2.96 | *2.36* | 3.15 | *2.51* | 3.34 |
| 18.6 | 2.80 | *2.23* | 2.90 | *2.31* | 3.10 | *2.47* | 3.30 | *2.63* | 3.48 |
| 18.8 | 2.95 | *2.35* | 3.05 | *2.43* | 3.25 | *2.59* | 3.45 | *2.75* | 3.63 |
| 19.0 | 3.10 | *2.47* | 3.19 | *2.54* | 3.40 | *2.71* | 3.59 | *2.86* | 3.77 |
| 19.2 | 3.25 | *2.59* | 3.34 | *2.66* | 3.55 | *2.83* | 3.73 | *2.98* | 3.92 |
| 19.4 | 3.39 | *2.70* | 3.48 | *2.78* | 3.70 | *2.95* | 3.88 | *3.10* | 4.07 |
| 19.6 | 3.53 | *2.82* | 3.63 | *2.90* | 3.84 | *3.06* | 4.03 | *3.22* | 4.22 |
| 19.8 | 3.68 | *2.94* | 3.78 | *3.02* | 3.98 | *3.18* | 4.17 | *3.33* | 4.37 |
| 20.0 | 3.83 | *3.06* | 3.93 | *3.13* | 4.13 | *3.30* | 4.32 | *3.45* | 4.52 |
| 20.2 | 3.97 | *3.17* | 4.07 | *3.25* | 4.27 | *3.41* | 4.47 | *3.57* | 4.66 |
| 20.4 | 4.12 | *3.29* | 4.22 | *3.37* | 4.42 | *3.53* | 4.61 | *3.68* | 4.82 |
| 20.6 | 4.26 | *3.41* | 4.36 | *3.49* | 4.56 | *3.65* | 4.75 | *3.80* | 4.96 |
| 20.8 | 4.41 | *3.52* | 4.51 | *3.60* | 4.70 | *3.76* | 4.90 | *3.92* | 5.10 |
| 21.0 | 4.56 | *3.64* | 4.65 | *3.72* | 4.85 | *3.88* | 5.04 | *4.03* | 5.24 |
| 21.2 | 4.70 | *3.76* | 4.80 | *3.84* | 4.99 | *3.99* | 5.19 | *4.15* | 5.39 |
| 21.4 | 4.84 | *3.87* | 4.94 | *3.95* | 5.14 | *4.11* | 5.33 | *4.26* | 5.53 |
| 21.6 | 4.99 | *3.99* | 5.09 | *4.07* | 5.28 | *4.22* | 5.47 | *4.38* | 5.67 |
| 21.8 | 5.13 | *4.10* | 5.23 | *4.18* | 5.43 | *4.34* | 5.61 | *4.49* | 5.82 |

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[1] Calculated and arranged by B. H. St. John from data of Doroshevskii and Dvorzhanchik.

## *Alcohol table*—Continued

| 21° C. | 22° C. | | 23° C. | | 24° C. | | 25° C. | | |
|---|---|---|---|---|---|---|---|---|---|
| Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | SCALE READING |
| .... | .... | .... | .... | .... | .... | .... | 0.00 | 0.00 | 13.2 |
| .... | .... | .... | .... | .... | .... | .... | 0.18 | 0.14 | 13.4 |
| .... | .... | .... | .... | .... | 0.14 | 0.11 | 0.35 | 0.28 | 13.6 |
| .... | .... | .... | 0.10 | 0.08 | 0.31 | 0.25 | 0.53 | 0.42 | 13.8 |
| .... | 0.08 | 0.06 | 0.28 | 0.22 | 0.49 | 0.39 | 0.70 | 0.56 | 14.0 |
| 0.03 | 0.24 | 0.19 | 0.45 | 0.36 | 0.67 | 0.53 | 0.88 | 0.70 | 14.2 |
| 0.17 | 0.41 | 0.33 | 0.63 | 0.50 | 0.84 | 0.67 | 1.06 | 0.85 | 14.4 |
| 0.30 | 0.59 | 0.47 | 0.80 | 0.64 | 1.02 | 0.81 | 1.24 | 0.99 | 14.6 |
| 0.44 | 0.77 | 0.61 | 0.98 | 0.78 | 1.19 | 0.95 | 1.40 | 1.11 | 14.8 |
| 0.58 | 0.94 | 0.75 | 1.16 | 0.92 | 1.36 | 1.08 | 1.55 | 1.23 | 15.0 |
| 0.72 | 1.12 | 0.89 | 1.32 | 1.05 | 1.51 | 2.20 | 1.71 | 1.36 | 15.2 |
| 0.85 | 1.29 | 1.02 | 1.47 | 1.17 | 1.66 | 1.32 | 1.86 | 1.48 | 15.4 |
| 0.99 | 1.44 | 1.15 | 1.62 | 1.29 | 1.82 | 1.44 | 2.01 | 1.60 | 15.6 |
| 1.11 | 1.60 | 1.27 | 1.77 | 1.41 | 1.97 | 1.57 | 2.17 | 1.72 | 15.8 |
| 1.23 | 1.75 | 1.39 | 1.92 | 1.53 | 2.12 | 1.69 | 2.33 | 1.85 | 16.0 |
| 1.35 | 1.90 | 1.51 | 2.08 | 1.65 | 2.27 | 1.81 | 2.48 | 1.97 | 16.2 |
| 1.47 | 2.05 | 1.63 | 2.24 | 1.78 | 2.43 | 1.93 | 2.62 | 2.09 | 16.4 |
| 1.59 | 2.20 | 1.75 | 2.39 | 1.90 | 2.57 | 2.05 | 2.77 | 2.21 | 16.6 |
| 1.71 | 2.35 | 1.87 | 2.53 | 2.02 | 2.72 | 2.17 | 2.92 | 2.33 | 16.8 |
| 1.83 | 2.50 | 1.99 | 2.69 | 2.14 | 2.87 | 2.29 | 3.06 | 2.44 | 17.0 |
| 1.95 | 2.65 | 2.11 | 2.82 | 2.25 | 3.02 | 2.41 | 3.21 | 2.56 | 17.2 |
| 2.07 | 2.79 | 2.23 | 2.97 | 2.37 | 3.17 | 2.53 | 3.36 | 2.68 | 17.4 |
| 2.18 | 2.94 | 2.34 | 3.12 | 2.49 | 3.32 | 2.65 | 3.51 | 2.80 | 17.6 |
| 2.30 | 3.09 | 2.46 | 3.27 | 2.61 | 3.46 | 2.76 | 3.66 | 2.92 | 17.8 |
| 2.42 | 3.23 | 2.58 | 3.42 | 2.73 | 3.61 | 2.88 | 3.81 | 3.04 | 18.0 |
| 2.54 | 3.37 | 2.69 | 3.57 | 2.85 | 3.76 | 3.00 | 3.96 | 3.16 | 18.2 |
| 2.66 | 3.52 | 2.81 | 3.71 | 2.96 | 3.91 | 3.12 | 4.11 | 3.28 | 18.4 |
| 2.78 | 3.66 | 2.92 | 3.86 | 3.08 | 4.06 | 3.24 | 4.26 | 3.40 | 18.6 |
| 2.89 | 3.81 | 3.04 | 4.01 | 3.20 | 4.21 | 3.36 | 4.41 | 3.52 | 18.8 |
| 3.01 | 3.96 | 3.16 | 4.16 | 3.32 | 4.36 | 3.48 | 4.56 | 3.64 | 19.0 |
| 3.13 | 4.11 | 3.28 | 4.31 | 3.44 | 4.51 | 3.60 | 4.70 | 3.76 | 19.2 |
| 3.25 | 4.26 | 3.40 | 4.46 | 3.56 | 4.65 | 3.72 | 4.85 | 3.88 | 19.4 |
| 3.37 | 4.41 | 3.52 | 4.61 | 3.68 | 4.80 | 3.84 | 5.00 | 4.00 | 19.6 |
| 3.49 | 4.56 | 3.64 | 4.75 | 3.80 | 4.95 | 3.96 | 5.15 | 4.12 | 19.8 |
| 3.61 | 4.72 | 3.77 | 4.90 | 3.92 | 5.10 | 4.08 | 5.29 | 4.23 | 20.0 |
| 3.73 | 4.87 | 3.89 | 5.05 | 4.04 | 5.24 | 4.19 | 5.44 | 4.35 | 20.2 |
| 3.85 | 5.01 | 4.01 | 5.20 | 4.16 | 5.38 | 4.31 | 5.58 | 4.47 | 20.4 |
| 3.97 | 5.15 | 4.12 | 5.34 | 4.27 | 5.52 | 4.42 | 5.72 | 4.58 | 20.6 |
| 4.08 | 5.29 | 4.24 | 5.48 | 4.39 | 5.67 | 4.54 | 5.87 | 4.70 | 20.8 |
| 4.20 | 5.44 | 4.35 | 5.62 | 4.50 | 5.82 | 4.66 | 6.02 | 4.81 | 21.0 |
| 4.31 | 5.58 | 4.47 | 5.77 | 4.62 | 5.96 | 4.77 | 6.16 | 4.93 | 21.2 |
| 4.43 | 5.72 | 4.58 | 5.91 | 4.73 | 6.11 | 4.89 | 6.30 | 5.05 | 21.4 |
| 4.54 | 5.87 | 4.70 | 6.06 | 4.85 | 6.25 | 5.01 | 6.44 | 5.16 | 21.6 |
| 4.66 | 6.01 | 4.81 | 6.20 | 4.97 | 6.39 | 5.12 | 6.59 | 5.28 | 21.8 |

22

*Alcohol table.*—Continued.

| SCALE READING | 17.5° C. | | 18° C. | | 19° C. | | 20° C. | | 21° C. |
|---|---|---|---|---|---|---|---|---|---|
| | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume |
| 22.0 | 5.27 | *4.22* | 5.37 | *4.30* | 5.57 | *4.45* | 5.76 | *4.61* | 5.96 |
| 22.2 | 5.41 | *4.33* | 5.51 | *4.41* | 5.71 | *4.57* | 5.90 | *4.72* | 6.11 |
| 22.4 | 5.56 | *4.45* | 5.65 | *4.53* | 5.85 | *4.68* | 6.05 | *4.84* | 6.25 |
| 22.6 | 5.70 | *4.56* | 5.80 | *4.64* | 6.00 | *4.80* | 3.19 | *4.95* | 6.39 |
| 22.8 | 5.85 | *4.68* | 5.94 | *4.75* | 6.14 | *4.91* | 6.33 | *5.07* | 6.53 |
| 23.0 | 5.99 | *4.79* | 6.08 | *4.87* | 6.28 | *5.03* | 6.47 | *5.18* | 6.67 |
| 23.2 | 6.13 | *4.91* | 6.22 | *4.98* | 6.42 | *5.14* | 6.61 | *5.30* | 6.81 |
| 23.4 | 6.27 | *5.02* | 6.36 | *5.10* | 6.56 | *5.26* | 6.75 | *5.41* | 6.95 |
| 23.6 | 6.41 | *5.14* | 6.50 | *5.21* | 6.70 | *5.37* | 6.90 | *5.53* | 7.09 |
| 23.8 | 6.55 | *5.25* | 6.64 | *5.32* | 6.85 | *5.49* | 7.04 | *5.64* | 7.23 |
| 24.0 | 6.69 | *5.36* | 6.78 | *5.44* | 6.99 | *5.60* | 7.18 | *5.76* | 7.38 |
| 24.2 | 6.83 | *5.47* | 6.92 | *5.55* | 7.13 | *5.71* | 7.32 | *5.87* | 7.52 |
| 24.4 | 6.97 | *5.59* | 7.06 | *5.66* | 7.27 | *5.83* | 7.46 | *5.99* | 7.66 |
| 24.6 | 7.11 | *5.70* | 7.20 | *5.78* | 7.41 | *5.94* | 7.60 | *6.10* | 7.80 |
| 24.8 | 7.25 | *5.81* | 7.35 | *5.89* | 7.55 | *6.06* | 7.74 | *6.21* | 7.93 |
| 25.0 | 7.39 | *5.93* | 7.49 | *6.01* | 7.68 | *6.16* | 7.88 | *6.32* | 8.06 |
| 25.2 | 7.53 | *6.04* | 7.63 | *6.12* | 7.82 | *6.27* | 8.01 | *6.43* | 8.20 |
| 25.4 | 7.66 | *6.15* | 7.76 | *6.23* | 7.95 | *6.38* | 8.14 | *6.54* | 8.34 |
| 25.6 | 7.80 | *6.26* | 7.90 | *6.34* | 8.09 | *6.49* | 8.28 | *6.65* | 8.48 |
| 25.8 | 7.94 | *6.37* | 8.03 | *6.44* | 8.22 | *6.60* | 8.42 | *6.76* | 8.62 |
| 26.0 | 8.07 | *6.48* | 8.16 | *6.55* | 8.36 | *6.71* | 8.55 | *6.87* | 8.75 |
| 26.2 | 8.21 | *6.59* | 8.30 | *6.66* | 8.50 | *6.82* | 8.69 | *6.98* | 8.89 |
| 26.4 | 8.34 | *6.70* | 8.44 | *6.78* | 8.63 | *6.93* | 8.82 | *7.09* | 9.03 |
| 26.6 | 8.48 | *6.81* | 8.57 | *6.88* | 8.77 | *7.04* | 8.96 | *7.20* | 9.16 |
| 26.8 | 8.62 | *6.92* | 8.71 | *6.99* | 8.91 | *7.15* | 9.10 | *7.31* | 9.30 |
| 27.0 | 8.75 | *7.03* | 8.85 | *7.11* | 9.05 | *7.27* | 9.23 | *7.42* | 9.44 |
| 27.2 | 8.89 | *7.14* | 8.98 | *7.22* | 9.18 | *7.38* | 9.37 | *7.54* | 9.58 |
| 27.4 | 9.02 | *7.25* | 9.12 | *7.33* | 9.32 | *7.49* | 9.51 | *7.65* | 9.71 |
| 27.6 | 9.16 | *7.36* | 9.26 | *7.44* | 9.45 | *7.60* | 9.65 | *7.76* | 9.85 |
| 27.8 | 9.29 | *7.47* | 9.39 | *7.55* | 9.59 | *7.71* | 9.79 | *7.87* | 9.98 |
| 28.0 | 9.43 | *7.58* | 9.53 | *7.66* | 9.72 | *7.82* | 9.92 | *7.98* | 10.12 |
| 28.2 | 9.57 | *7.69* | 9.66 | *7.77* | 9.86 | *7.93* | 10.06 | *8.09* | 10.25 |
| 28.4 | 9.70 | *7.80* | 9.80 | *7.88* | 9.99 | *8.04* | 10.19 | *8.20* | 10.39 |
| 28.6 | 9.84 | *7.91* | 9.93 | *7.99* | 10.13 | *8.15* | 10.32 | *8.31* | 10.52 |
| 28.8 | 9.97 | *8.02* | 10.07 | *8.10* | 10.26 | *8.26* | 10.46 | *8.42* | 10.66 |
| 29.0 | 10.10 | *8.13* | 10.19 | *8.20* | 10.40 | *8.36* | 10.59 | *8.53* | 10.79 |
| 29.2 | 10.24 | *8.24* | 10.33 | *8.31* | 10.52 | *8.47* | 10.73 | *8.64* | 10.93 |
| 29.4 | 10.36 | *8.34* | 10.46 | *8.42* | 10.66 | *8.58* | 10.86 | *8.74* | 11.06 |
| 29.6 | 10.50 | *8.45* | 10.59 | *8.53* | 10.79 | *8.69* | 10.99 | *8.85* | 11.20 |
| 29.8 | 10.63 | *8.56* | 10.72 | *8.63* | 10.93 | *8.80* | 11.12 | *8.96* | 11.33 |
| 30.0 | 10.76 | *8.66* | 10.86 | *8.74* | 11.05 | *8.91* | 11.26 | *9.07* | 11.46 |
| 30.2 | 10.89 | *8.77* | 10.99 | *8.85* | 11.18 | *9.02* | 11.38 | *9.18* | 11.59 |
| 30.4 | 11.02 | *8.88* | 11.12 | *8.96* | 11.31 | *9.12* | 11.51 | *9.28* | 11.72 |
| 30.6 | 11.15 | *8.98* | 11.25 | *9.06* | 11.44 | *9.23* | 11.64 | *9.39* | 11.85 |
| 30.8 | 11.28 | *9.09* | 11.38 | *9.17* | 11.58 | *9.34* | 11.78 | *9.50* | 11.99 |

*Alcohol table.*—Continued.

| 21° C. | 22° C. | | 23° C. | | 24° C. | | 25° C. | | |
|---|---|---|---|---|---|---|---|---|---|
| Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | SCALE READING |
| 4.77 | 6.15 | 4.93 | 6.34 | 5.08 | 6.54 | 5.24 | 6.73 | 5.39 | 22.0 |
| 4.89 | 6.29 | 5.04 | 6.49 | 5.20 | 6.68 | 5.35 | 6.87 | 5.51 | 22.2 |
| 5.00 | 6.43 | 5.15 | 6.63 | 5.31 | 6.82 | 5.47 | 7.01 | 5.62 | 22.4 |
| 5.11 | 6.57 | 5.27 | 6.77 | 5.43 | 6.96 | 5.58 | 7.16 | 5.74 | 22.6 |
| 5.23 | 6.71 | 5.38 | 6.91 | 5.54 | 7.10 | 5.70 | 7.31 | 5.86 | 22.8 |
| 5.34 | 6.86 | 5.50 | 7.06 | 5.66 | 7.24 | 5.81 | 7.45 | 5.97 | 23.0 |
| 5.46 | 7.00 | 5.61 | 7.20 | 5.77 | 7.39 | 5.93 | 7.59 | 6.08 | 23.2 |
| 5.57 | 7.14 | 5.73 | 7.34 | 5.89 | 7.53 | 6.04 | 7.73 | 6.20 | 23.4 |
| 5.69 | 7.28 | 5.84 | 7.48 | 6.00 | 7.67 | 6.15 | 7.87 | 6.31 | 23.6 |
| 5.80 | 7.42 | 5.95 | 7.62 | 6.11 | 7.81 | 6.27 | 8.00 | 6.42 | 23.8 |
| 5.92 | 7.56 | 6.07 | 7.76 | 6.22 | 7.95 | 6.38 | 8.14 | 6.53 | 24.0 |
| 6.03 | 7.70 | 6.18 | 7.90 | 6.34 | 8.09 | 6.49 | 8.28 | 6.65 | 24.2 |
| 6.14 | 7.84 | 6.29 | 8.04 | 6.45 | 8.23 | 6.60 | 8.42 | 6.76 | 24.4 |
| 6.25 | 7.98 | 6.41 | 8.17 | 6.56 | 8.37 | 6.72 | 8.55 | 6.87 | 24.6 |
| 6.36 | 8.12 | 6.52 | 8.31 | 6.67 | 8.51 | 6.83 | 8.69 | 6.98 | 24.8 |
| 6.47 | 8.26 | 6.63 | 8.45 | 6.79 | 8.64 | 6.94 | 8.84 | 7.10 | 25.0 |
| 6.59 | 8.40 | 6.75 | 8.59 | 6.90 | 8.78 | 7.06 | 8.98 | 7.21 | 25.2 |
| 6.70 | 8.54 | 6.86 | 8.73 | 7.01 | 8.92 | 7.17 | 9.12 | 7.33 | 25.4 |
| 6.81 | 8.68 | 6.97 | 8.86 | 7.12 | 9.06 | 7.28 | 9.26 | 7.44 | 25.6 |
| 6.92 | 8.82 | 7.08 | 9.00 | 7.23 | 9.20 | 7.40 | 9.39 | 7.55 | 25.8 |
| 7.03 | 8.95 | 7.19 | 9.14 | 7.35 | 9.34 | 7.51 | 9.53 | 7.67 | 26.0 |
| 7.14 | 9.09 | 7.30 | 9.28 | 7.46 | 9.48 | 7.62 | 9.67 | 7.78 | 26.2 |
| 7.25 | 9.22 | 7.41 | 9.42 | 7.57 | 9.61 | 7.73 | 9.81 | 7.90 | 26.4 |
| 7.36 | 9.36 | 7.52 | 9.55 | 7.68 | 9.75 | 7.85 | 9.95 | 8.01 | 26.6 |
| 7.47 | 9.49 | 7.63 | 9.69 | 7.79 | 9.89 | 7.96 | 10.09 | 8.12 | 26.8 |
| 7.59 | 9.63 | 7.74 | 9.83 | 7.91 | 10.03 | 8.07 | 10.23 | 8.24 | 27.0 |
| 7.70 | 9.76 | 7.85 | 9.97 | 8.02 | 10.17 | 8.18 | 10.37 | 8.35 | 27.2 |
| 7.81 | 9.90 | 7.96 | 10.10 | 8.13 | 10.31 | 8.30 | 10.51 | 8.46 | 27.4 |
| 7.92 | 10.03 | 8.07 | 10.24 | 8.24 | 10.45 | 8.41 | 10.65 | 8.57 | 27.6 |
| 8.03 | 10.17 | 8.18 | 10.38 | 8.35 | 10.58 | 8.52 | 10.79 | 8.69 | 27.8 |
| 8.14 | 10.31 | 8.30 | 10.51 | 8.46 | 10.72 | 8.63 | 10.93 | 8.80 | 28.0 |
| 8.25 | 10.45 | 8.41 | 10.65 | 8.58 | 10.86 | 8.74 | 11.06 | 8.91 | 28.2 |
| 8.36 | 10.59 | 8.52 | 10.79 | 8.69 | 11.00 | 8.86 | 11.20 | 9.02 | 28.4 |
| 8.47 | 10.72 | 8.63 | 10.93 | 8.80 | 11.13 | 8.97 | 11.33 | 9.13 | 28.6 |
| 8.58 | 10.86 | 8.74 | 11.06 | 8.91 | 11.27 | 9.08 | 11.47 | 9.24 | 28.8 |
| 8.69 | 11.00 | 8.86 | 11.20 | 9.02 | 11.40 | 9.19 | 11.61 | 9.36 | 29.0 |
| 8.80 | 11.13 | 8.97 | 11.33 | 9.13 | 11.54 | 9.30 | 11.75 | 9.47 | 29.2 |
| 8.91 | 11.27 | 9.08 | 11.47 | 9.24 | 11.67 | 9.41 | 11.88 | 9.58 | 29.4 |
| 9.02 | 11.39 | 9.18 | 11.60 | 9.35 | 11.81 | 9.52 | 12.01 | 9.69 | 29.6 |
| 9.13 | 11.53 | 9.29 | 11.74 | 9.46 | 11.94 | 9.63 | 12.15 | 9.80 | 29.8 |
| 9.24 | 11.66 | 9.40 | 11.87 | 9.57 | 12.08 | 9.74 | 12.29 | 9.91 | 30.0 |
| 9.34 | 11.79 | 9.51 | 12.00 | 9.68 | 12.21 | 9.85 | 12.42 | 10.02 | 30.2 |
| 9.45 | 11.93 | 9.62 | 12.13 | 9.79 | 12.34 | 9.96 | 12.56 | 10.13 | 30.4 |
| 9.56 | 12.06 | 9.73 | 12.27 | 9.90 | 12.48 | 10.07 | 12.70 | 10.24 | 30.6 |
| 9.67 | 12.19 | 9.84 | 12.40 | 10.01 | 12.61 | 10.18 | 12.84 | 10.36 | 30.8 |

**22** *Alcohol table.*—Continued.

| SCALE READING | 17.5° C. | | 18° C. | | 19° C. | | 20° C. | | 21° C. |
|---|---|---|---|---|---|---|---|---|---|
| | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume |
| 31.0 | 11.41 | 9.19 | 11.51 | 9.28 | 11.71 | 9.44 | 11.91 | 9.60 | 12.12 |
| 31.2 | 11.54 | 9.30 | 11.64 | 9.38 | 11.84 | 9.55 | 12.04 | 9.71 | 12.25 |
| 31.4 | 11.66 | 9.40 | 11.77 | 9.49 | 11.97 | 9.65 | 12.17 | 9.82 | 12.38 |
| 31.6 | 11.79 | 9.51 | 11.90 | 9.59 | 12.10 | 9.76 | 12.30 | 9.92 | 12.51 |
| 31.8 | 11.92 | 9.62 | 12.03 | 9.70 | 12.23 | 9.87 | 12.43 | 10.03 | 12.64 |
| 32.0 | 12.05 | 9.72 | 12.15 | 9.80 | 12.36 | 9.97 | 12.57 | 10.14 | 12.78 |
| 32.2 | 12.18 | 9.83 | 12.28 | 9.91 | 12.49 | 10.08 | 12.70 | 10.25 | 12.91 |
| 32.4 | 12.31 | 9.93 | 12.40 | 10.02 | 12.62 | 10.19 | 12.83 | 10.35 | 13.04 |
| 32.6 | 12.43 | 10.04 | 12.54 | 10.12 | 12.75 | 10.29 | 12.96 | 10.46 | 13.17 |
| 32.8 | 12.56 | 10.14 | 12.67 | 10.23 | 12.88 | 10.40 | 13.09 | 10.57 | 13.30 |
| 33.0 | 12.69 | 10.24 | 12.79 | 10.33 | 13.01 | 10.50 | 13.22 | 10.68 | 13.43 |
| 33.2 | 12.82 | 10.35 | 12.92 | 10.43 | 13.13 | 10.61 | 13.35 | 10.79 | 13.56 |
| 33.4 | 12.95 | 10.45 | 13.05 | 10.54 | 13.26 | 10.71 | 13.48 | 10.89 | 13.69 |
| 33.6 | 13.08 | 10.56 | 13.18 | 10.64 | 13.39 | 10.82 | 13.61 | 11.00 | 13.82 |
| 33.8 | 13.20 | 10.66 | 13.30 | 10.75 | 13.52 | 10.93 | 13.74 | 11.10 | 13.95 |
| 34.0 | 13.33 | 10.77 | 13.43 | 10.85 | 13.64 | 11.03 | 13.86 | 11.21 | 14.08 |
| 34.2 | 13.45 | 10.87 | 13.56 | 10.96 | 13.77 | 11.13 | 13.99 | 11.31 | 14.21 |
| 34.4 | 13.58 | 10.97 | 13.68 | 11.06 | 13.90 | 11.24 | 14.12 | 11.41 | 14.34 |
| 34.6 | 13.70 | 11.08 | 13.81 | 11.16 | 14.02 | 11.34 | 14.25 | 11.52 | 14.47 |
| 34.8 | 13.83 | 11.18 | 13.94 | 11.27 | 14.14 | 11.44 | 14.37 | 11.62 | 14.59 |
| 35.0 | 13.96 | 11.28 | 14.06 | 11.37 | 14.27 | 11.55 | 14.50 | 11.73 | 14.72 |
| 35.2 | 14.08 | 11.38 | 14.19 | 11.47 | 14.39 | 11.65 | 14.62 | 11.83 | 14.85 |
| 35.4 | 14.21 | 11.49 | 14.31 | 11.57 | 14.52 | 11.75 | 14.75 | 11.93 | 14.97 |
| 35.6 | 14.33 | 11.59 | 14.44 | 11.68 | 14.65 | 11.86 | 14.87 | 12.04 | 15.10 |
| 35.8 | 14.46 | 11.69 | 14.56 | 11.78 | 14.78 | 11.96 | 15.00 | 12.14 | 15.23 |
| 36.0 | 14.58 | 11.79 | 14.69 | 11.88 | 14.90 | 12.06 | 15.13 | 12.24 | 15.35 |
| 36.2 | 14.71 | 11.90 | 14.81 | 11.99 | 15.03 | 12.16 | 15.25 | 12.35 | 15.48 |
| 36.4 | 14.83 | 12.00 | 14.94 | 12.09 | 15.16 | 12.27 | 15.38 | 12.45 | 15.61 |
| 36.6 | 14.96 | 12.10 | 15.06 | 12.19 | 15.28 | 12.37 | 15.51 | 12.56 | 15.73 |
| 36.8 | 15.08 | 12.20 | 15.19 | 12.29 | 15.41 | 12.47 | 15.63 | 12.66 | 15.86 |
| 37.0 | 15.20 | 12.30 | 15.31 | 12.40 | 15.53 | 12.58 | 15.76 | 12.77 | 15.99 |
| 37.2 | 15.33 | 12.41 | 15.44 | 12.50 | 15.66 | 12.68 | 15.89 | 12.87 | 16.11 |
| 37.4 | 15.45 | 12.51 | 15.56 | 12.60 | 15.79 | 12.78 | 16.01 | 12.97 | 16.24 |
| 37.6 | 15.57 | 12.61 | 15.69 | 12.70 | 15.91 | 12.89 | 16.14 | 13.08 | 16.37 |
| 37.8 | 15.70 | 12.71 | 15.81 | 12.81 | 16.04 | 12.99 | 16.26 | 13.18 | 16.49 |
| 38.0 | 15.82 | 12.81 | 15.94 | 12.91 | 16.16 | 13.09 | 16.39 | 13.28 | 16.62 |
| 38.2 | 15.94 | 12.91 | 16.06 | 13.01 | 16.29 | 13.19 | 16.51 | 13.38 | 16.75 |
| 38.4 | 16.07 | 13.02 | 16.18 | 13.11 | 16.41 | 13.30 | 16.64 | 13.49 | 16.87 |
| 38.6 | 16.19 | 13.12 | 16.31 | 13.21 | 16.53 | 13.40 | 16.76 | 13.59 | 17.00 |
| 38.8 | 16.31 | 13.22 | 16.43 | 13.31 | 16.66 | 13.50 | 16.89 | 13.69 | 17.13 |
| 39.0 | 16.44 | 13.32 | 16.55 | 13.42 | 16.78 | 13.61 | 17.01 | 13.79 | 17.25 |
| 39.2 | 16.56 | 13.42 | 16.67 | 13.52 | 16.91 | 13.71 | 17.14 | 13.90 | 17.38 |
| 39.4 | 16.68 | 13.52 | 16.80 | 13.62 | 17.03 | 13.81 | 17.26 | 14.00 | 17.50 |
| 39.6 | 16.80 | 13.62 | 16.92 | 13.72 | 17.15 | 13.91 | 17.39 | 14.10 | 17.63 |
| 39.8 | 16.93 | 13.73 | 17.04 | 13.82 | 17.28 | 14.02 | 17.51 | 14.21 | 17.75 |

*Alcohol table.*—Continued.

| 21° C. | 22° C. | | 23° C. | | 24° C. | | 25° C. | | |
|---|---|---|---|---|---|---|---|---|---|
| Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | SCALE READING |
| 9.77 | 12.32 | 9.95 | 12.54 | 10.12 | 12.75 | 10.29 | 12.97 | 10.47 | 31.0 |
| 9.88 | 12.46 | 10.05 | 12.67 | 10.23 | 12.89 | 10.40 | 13.11 | 10.58 | 31.2 |
| 9.99 | 12.59 | 10.16 | 12.81 | 10.34 | 13.02 | 10.51 | 13.24 | 10.69 | 31.4 |
| 10.10 | 12.72 | 10.27 | 12.94 | 10.45 | 13.15 | 10.62 | 13.37 | 10.80 | 31.6 |
| 10.21 | 12.85 | 10.38 | 13.07 | 10.55 | 13.29 | 10.73 | 13.51 | 10.91 | 31.8 |
| 10.31 | 12.99 | 10.49 | 13.20 | 10.66 | 13.42 | 10.84 | 13.64 | 11.02 | 32.0 |
| 10.42 | 13.12 | 10.59 | 13.34 | 10.77 | 13.55 | 10.95 | 13.77 | 11.13 | 32.2 |
| 10.53 | 13.25 | 10.70 | 13.47 | 10.88 | 13.69 | 11.06 | 13.91 | 11.24 | 32.4 |
| 10.64 | 13.38 | 10.81 | 13.60 | 10.99 | 13.82 | 11.17 | 14.04 | 11.35 | 32.6 |
| 10.75 | 13.51 | 10.92 | 13.73 | 11.10 | 13.95 | 11.28 | 14.17 | 11.46 | 32.8 |
| 10.85 | 13.64 | 11.03 | 13.85 | 11.21 | 14.09 | 11.39 | 14.31 | 11.57 | 33.0 |
| 10.96 | 13.78 | 11.13 | 13.99 | 11.31 | 14.22 | 11.49 | 14.44 | 11.68 | 33.2 |
| 11.07 | 13.91 | 11.24 | 14.13 | 11.42 | 14.35 | 11.60 | 14.58 | 11.79 | 33.4 |
| 11.17 | 14.04 | 11.35 | 14.26 | 11.53 | 14.48 | 11.71 | 14.71 | 11.90 | 33.6 |
| 11.28 | 14.17 | 11.45 | 14.39 | 11.64 | 14.62 | 11.82 | 14.85 | 12.01 | 33.8 |
| 11.38 | 14.30 | 11.56 | 14.52 | 11.75 | 14.75 | 11.93 | 14.98 | 12.12 | 34.0 |
| 11.49 | 14.43 | 11.67 | 14.65 | 11.85 | 14.88 | 12.04 | 15.11 | 12.23 | 34.2 |
| 11.59 | 14.57 | 11.78 | 14.78 | 11.96 | 15.01 | 12.15 | 15.25 | 12.34 | 34.4 |
| 11.70 | 14.70 | 11.88 | 14.91 | 12.07 | 15.14 | 12.26 | 15.38 | 12.45 | 34.6 |
| 11.81 | 14.83 | 11.99 | 15.05 | 12.18 | 15.28 | 12.36 | 15.51 | 12.56 | 34.8 |
| 11.91 | 14.96 | 12.10 | 15.18 | 12.28 | 15.41 | 12.47 | 15.65 | 12.67 | 35.0 |
| 12.02 | 15.09 | 12.20 | 15.31 | 12.39 | 15.54 | 12.58 | 15.78 | 12.78 | 35.2 |
| 12.12 | 15.22 | 12.31 | 15.44 | 12.50 | 15.67 | 12.69 | 15.91 | 12.89 | 35.4 |
| 12.23 | 15.34 | 12.41 | 15.56 | 12.60 | 15.80 | 12.80 | 16.05 | 13.00 | 35.6 |
| 12.33 | 15.47 | 12.52 | 15.69 | 12.71 | 15.93 | 12.91 | 16.18 | 13.11 | 35.8 |
| 12.43 | 15.59 | 12.62 | 15.82 | 12.82 | 16.06 | 13.02 | 16.31 | 13.21 | 36.0 |
| 12.54 | 15.72 | 12.73 | 15.95 | 12.92 | 16.19 | 13.12 | 16.44 | 13.32 | 36.2 |
| 12.64 | 15.85 | 12.84 | 16.08 | 13.03 | 16.32 | 13.23 | 16.56 | 13.43 | 36.4 |
| 12.75 | 15.97 | 12.94 | 16.21 | 13.14 | 16.45 | 13.34 | 16.69 | 13.53 | 36.6 |
| 12.85 | 16.10 | 13.05 | 16.34 | 13.24 | 16.58 | 13.44 | 16.82 | 13.64 | 36.8 |
| 12.96 | 16.23 | 13.15 | 16.47 | 13.35 | 16.71 | 13.55 | 16.95 | 13.75 | 37.0 |
| 13.06 | 16.35 | 13.26 | 16.60 | 13.45 | 16.84 | 13.65 | 17.08 | 13.86 | 37.2 |
| 13.16 | 16.48 | 13.36 | 16.72 | 13.56 | 16.97 | 13.76 | 17.21 | 13.96 | 37.4 |
| 13.27 | 16.61 | 13.46 | 16.85 | 13.66 | 17.09 | 13.87 | 17.34 | 14.07 | 37.6 |
| 13.37 | 16.73 | 13.57 | 16.98 | 13.77 | 17.22 | 13.97 | 17.46 | 14.17 | 37.8 |
| 13.47 | 16.86 | 13.67 | 17.11 | 13.87 | 17.35 | 14.08 | 17.59 | 14.28 | 38.0 |
| 13.58 | 16.99 | 13.78 | 17.23 | 13.98 | 17.47 | 14.18 | 17.72 | 14.38 | 38.2 |
| 13.68 | 17.11 | 13.88 | 17.36 | 14.08 | 17.60 | 14.29 | 17.85 | 14.49 | 38.4 |
| 13.79 | 17.24 | 13.98 | 17.48 | 14.19 | 17.73 | 14.39 | 17.97 | 14.59 | 38.6 |
| 13.89 | 17.36 | 14.09 | 17.61 | 14.29 | 17.85 | 14.49 | 18.10 | 14.70 | 38.8 |
| 13.99 | 17.49 | 14.19 | 17.74 | 14.40 | 17.98 | 14.60 | 18.23 | 14.80 | 39.0 |
| 14.10 | 17.62 | 14.30 | 17.86 | 14.50 | 18.11 | 14.70 | 18.35 | 14.91 | 39.2 |
| 14.20 | 17.74 | 14.40 | 17.99 | 14.60 | 18.23 | 14.81 | 18.48 | 15.01 | 39.4 |
| 14.30 | 17.87 | 14.50 | 18.11 | 14.71 | 18.36 | 14.91 | 18.61 | 15.12 | 39.6 |
| 14.41 | 17.99 | 14.61 | 18.24 | 14.81 | 18.48 | 15.02 | 18.73 | 15.22 | 39.8 |

22 *Alcohol table.*—Continued.

| SCALE READING | 17.5° C. | | 18° C. | | 19° C. | | 20° C. | | 21° C. |
|---|---|---|---|---|---|---|---|---|---|
| | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume |
| 40.0 | 17.05 | *13.83* | 17.16 | *13.92* | 17.40 | *14.12* | 17.63 | *14.31* | 17.88 |
| 40.2 | 17.17 | *13.93* | 17.29 | *14.03* | 17.52 | *14.22* | 17.76 | *14.41* | 18.00 |
| 40.4 | 17.29 | *14.03* | 17.41 | *14.13* | 17.64 | *14.32* | 17.88 | *14.51* | 18.12 |
| 40.6 | 17.41 | *14.13* | 17.53 | *14.23* | 17.77 | *14.42* | 18.01 | *14.62* | 18.25 |
| 40.8 | 17.54 | *14.23* | 17.65 | *14.33* | 17.89 | *14.52* | 18.13 | *14.72* | 18.37 |
| 41.0 | 17.66 | *14.33* | 17.77 | *14.43* | 18.01 | *14.62* | 18.25 | *14.82* | 18.49 |
| 41.2 | 17.78 | *14.43* | 17.90 | *14.53* | 18.13 | *14.73* | 18.37 | *14.92* | 18.62 |
| 41.4 | 17.90 | *14.53* | 18.03 | *14.63* | 18.26 | *14.83* | 18.50 | *15.03* | 18.74 |
| 41.6 | 18.02 | *14.63* | 18.14 | *14.73* | 18.38 | *14.93* | 18.62 | *15.13* | 18.86 |
| 41.8 | 18.14 | *14.73* | 18.26 | *14.83* | 18.50 | *15.03* | 18.74 | *15.23* | 18.99 |
| 42.0 | 18.27 | *14.83* | 18.38 | *14.93* | 18.62 | *15.13* | 18.87 | *15.33* | 19.11 |
| 42.2 | 18.39 | *14.93* | 18.50 | *15.03* | 18.74 | *15.23* | 18.99 | *15.43* | 19.23 |
| 42.4 | 18.51 | *15.03* | 18.62 | *15.13* | 18.87 | *15.33* | 19.11 | *15.53* | 19.36 |
| 42.6 | 18.63 | *15.13* | 18.75 | *15.23* | 18.99 | *15.43* | 19.23 | *15.63* | 19.48 |
| 42.8 | 18.75 | *15.23* | 18.87 | *15.33* | 19.11 | *15.53* | 19.36 | *15.74* | 19.60 |
| 43.0 | 18.87 | *15.33* | 18.99 | *15.43* | 19.23 | *15.63* | 19.48 | *15.84* | 19.72 |
| 43.2 | 18.99 | *15.43* | 19.11 | *15.53* | 19.35 | *15.73* | 19.60 | *15.94* | 19.85 |
| 43.4 | 19.11 | *15.53* | 19.23 | *15.63* | 19.47 | *15.83* | 19.72 | *16.04* | 19.97 |
| 43.6 | 19.23 | *15.63* | 19.35 | *15.73* | 19.59 | *15.93* | 19.85 | *16.14* | 20.09 |
| 43.8 | 19.35 | *15.73* | 19.47 | *15.83* | 19.72 | *16.03* | 19.97 | *12.24* | 20.21 |
| 44.0 | 19.46 | *15.83* | 19.59 | *15.93* | 19.84 | *16.13* | 20.09 | *16.34* | 20.34 |
| 44.2 | 19.58 | *15.93* | 19.71 | *16.03* | 19.96 | *16.23* | 20.21 | *16.44* | 20.46 |
| 44.4 | 19.70 | *16.02* | 19.83 | *16.13* | 20.08 | *16.33* | 20.33 | *16.55* | 20.58 |
| 44.6 | 19.82 | *16.12* | 19.95 | *16.23* | 20.20 | *16.43* | 20.45 | *16.65* | 20.70 |
| 44.8 | 19.94 | *16.22* | 20.07 | *16.32* | 20.32 | *16.53* | 20.58 | *16.75* | 20.82 |
| 45.0 | 20.06 | *16.32* | 20.18 | *16.42* | 20.44 | *16.63* | 20.70 | *16.85* | 20.95 |
| 45.2 | 20.18 | *16.41* | 20.30 | *16.52* | 20.56 | *16.73* | 20.82 | *16.95* | 21.07 |
| 45.4 | 20.29 | *16.51* | 20.42 | *16.62* | 20.68 | *18.83* | 20.94 | *17.05* | 21.19 |
| 45.6 | 20.41 | *16.61* | 20.54 | *16.72* | 20.80 | *16.93* | 21.06 | *17.15* | 21.31 |
| 45.8 | 20.53 | *16.71* | 20.66 | *16.81* | 20.92 | *17.03* | 21.18 | *17.25* | 21.43 |
| 46.0 | 20.65 | *16.80* | 20.78 | *16.91* | 21.04 | *17.13* | 21.30 | *17.35* | 21.54 |
| 46.2 | 20.76 | *16.90* | 20.89 | *17.01* | 21.16 | *17.23* | 21.42 | *17.45* | 21.66 |
| 46.4 | 20.88 | *17.00* | 21.01 | *17.11* | 21.28 | *17.33* | 21.54 | *17.55* | 21.78 |
| 46.6 | 21.00 | *17.10* | 21.13 | *17.21* | 21.40 | *17.43* | 21.66 | *17.65* | 21.90 |
| 46.8 | 21.12 | *17.20* | 21.25 | *17.31* | 21.52 | *17.53* | 21.78 | *17.75* | 22.02 |
| 47.0 | 21.24 | *17.30* | 21.37 | *17.41* | 21.64 | *17.63* | 21.90 | *17.85* | 22.15 |
| 47.2 | 21.36 | *17.40* | 21.49 | *17.51* | 21.76 | *17.73* | 22.02 | *17.95* | 22.27 |
| 47.4 | 21.48 | *17.50* | 21.61 | *17.61* | 21.88 | *17.83* | 22.15 | *18.06* | 22.39 |
| 47.6 | 21.60 | *17.60* | 21.73 | *17.71* | 22.00 | *17.94* | 22.27 | *18.16* | 22.51 |
| 47.8 | 21.72 | *17.70* | 21.85 | *17.81* | 22.12 | *18.04* | 22.39 | *18.26* | 22.64 |
| 48.0 | 21.84 | *17.80* | 21.97 | *17.91* | 22.24 | *18.14* | 22.51 | *18.36* | 22.76 |
| 48.2 | 21.96 | *17.90* | 22.09 | *18.01* | 22.36 | *18.24* | 22.63 | *18.46* | 22.88 |
| 48.4 | 22.08 | *18.00* | 22.21 | *18.11* | 22.48 | *18.34* | 22.75 | *18.56* | 23.01 |
| 48.6 | 22.20 | *18.10* | 22.33 | *18.21* | 22.60 | *18.44* | 22.87 | *18.66* | 23.13 |
| 48.8 | 22.32 | *18.20* | 22.45 | *18.31* | 22.72 | *18.54* | 22.99 | *18.76* | 23.26 |

## *Alcohol table.*—Continued.

| 21° C. | 22° C. | | 23° C. | | 24° C. | | 25° C. | | SCALE READING |
|---|---|---|---|---|---|---|---|---|---|
| Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | |
| *14.51* | 18.12 | *14.71* | 18.36 | *14.92* | 18.61 | *15.12* | 18.86 | *15.32* | 40.0 |
| *14.61* | 18.24 | *14.82* | 18.49 | *15.02* | 18.74 | *15.22* | 18.99 | *15.43* | 40.2 |
| *14.72* | 18.37 | *14.92* | 18.61 | *15.12* | 18.86 | *15.33* | 19.11 | *15.53* | 40.4 |
| *14.82* | 18.49 | *15.03* | 18.74 | *15.23* | 18.99 | *15.43* | 19.24 | *15.64* | 40.6 |
| *14.92* | 18.61 | *15.13* | 18.86 | *15.33* | 19.11 | *15.53* | 19.37 | *15.74* | 40.8 |
| *15.03* | 18.74 | *15.23* | 18.99 | *15.43* | 19.24 | *15.64* | 19.49 | *15.85* | 41.0 |
| *15.13* | 18.86 | *15.33* | 19.11 | *15.53* | 19.36 | *15.74* | 19.62 | *15.95* | 41.2 |
| *15.23* | 18.99 | *15.43* | 19.24 | *15.64* | 19.49 | *15.84* | 19.75 | *16.06* | 41.4 |
| *15.33* | 19.11 | *15.53* | 19.36 | *15.74* | 19.61 | *15.95* | 19.87 | *16.16* | 41.6 |
| *15.43* | 19.23 | *15.63* | 19.48 | *15.84* | 19.74 | *16.05* | 20.00 | *16.27* | 41.8 |
| *15.53* | 19.36 | *15.74* | 19.61 | *15.94* | 19.86 | *16.16* | 20.13 | *16.37* | 42.0 |
| *15.63* | 19.48 | *15.84* | 19.73 | *16.05* | 19.99 | *16.26* | 20.25 | *16.48* | 42.2 |
| *15.74* | 19.60 | *15.94* | 19.86 | *16.15* | 20.11 | *16.36* | 20.38 | *16.58* | 42.4 |
| *15.84* | 19.72 | *16.04* | 19.98 | *16.25* | 20.24 | *16.47* | 20.50 | *16.69* | 42.6 |
| *15.94* | 19.85 | *16.14* | 20.10 | *16.35* | 20.36 | *16.57* | 20.63 | *16.79* | 42.8 |
| *16.04* | 19.97 | *16.24* | 20.23 | *16.46* | 20.49 | *16.67* | 20.75 | *16.90* | 43.0 |
| *16.14* | 20.09 | *16.35* | 20.35 | *16.56* | 20.61 | *16.78* | 20.88 | *17.00* | 43.2 |
| *16.24* | 20.21 | *16.45* | 20.47 | *16.66* | 20.74 | *16.88* | 21.01 | *17.10* | 43.4 |
| *16.34* | 20.34 | *16.55* | 20.60 | *16.76* | 20.86 | *16.98* | 21.13 | *17.21* | 43.6 |
| *16.45* | 20.46 | *16.65* | 20.72 | *16.87* | 20.99 | *17.09* | 21.25 | *17.31* | 43.8 |
| *16.55* | 20.58 | *16.76* | 20.84 | *16.97* | 21.11 | *17.19* | 21.38 | *17.41* | 44.0 |
| *16.65* | 20.71 | *16.86* | 20.96 | *17.07* | 21.23 | *17.29* | 21.50 | *17.52* | 44.2 |
| *16.75* | 20.83 | *16.96* | 21.09 | *17.17* | 21.36 | *17.40* | 21.63 | *17.62* | 44.4 |
| *16.85* | 20.95 | *17.06* | 21.21 | *17.28* | 21.48 | *17.50* | 21.75 | *17.73* | 44.6 |
| *16.95* | 21.07 | *17.16* | 21.33 | *17.38* | 21.60 | *17.60* | 21.88 | *17.83* | 44.8 |
| *17.06* | 21.19 | *17.26* | 21.45 | *17.48* | 21.73 | *17.71* | 22.00 | *17.93* | 45.0 |
| *17.16* | 21.31 | *17.36* | 21.58 | *17.58* | 21.85 | *17.81* | 22.13 | *18.04* | 45.2 |
| *17.26* | 21.43 | *17.46* | 21.70 | *17.68* | 21.98 | *17.91* | 22.25 | *18.14* | 45.4 |
| *17.36* | 21.55 | *17.56* | 21.82 | *17.79* | 22.10 | *18.02* | 22.38 | *18.25* | 45.6 |
| *17.46* | 21.67 | *17.66* | 21.94 | *17.89* | 22.23 | *18.12* | 22.51 | *18.36* | 45.8 |
| *17.56* | 21.79 | *17.76* | 22.07 | *17.99* | 22.35 | *18.23* | 22.64 | *18.47* | 46.0 |
| *18.66* | 21.91 | *17.86* | 22.19 | *18.09* | 22.48 | *18.33* | 22.76 | *18.57* | 46.2 |
| *17.76* | 22.03 | *17.96* | 22.32 | *18.20* | 22.61 | *18.44* | 22.89 | *18.68* | 46.4 |
| *17.86* | 22.16 | *18.06* | 22.44 | *18.30* | 22.73 | *18.55* | 23.02 | *18.79* | 46.6 |
| *17.96* | 22.28 | *18.17* | 22.57 | *18.41* | 22.86 | *18.65* | 23.15 | *18.90* | 46.8 |
| *18.06* | 22.41 | *18.27* | 22.69 | *18.51* | 22.99 | *18.76* | 23.28 | *19.00* | 47.0 |
| *18.16* | 22.53 | *18.38* | 22.82 | *18.62* | 23.12 | *18.86* | 23.41 | *19.11* | 47.2 |
| *18.26* | 22.66 | *18.48* | 22.94 | *18.72* | 23.24 | *18.97* | 23.54 | *19.22* | 47.4 |
| *18.36* | 22.78 | *18.58* | 23.07 | *18.83* | 23.37 | *19.08* | 23.67 | *19.33* | 47.6 |
| *18.47* | 22.91 | *18.69* | 23.20 | *18.93* | 23.50 | *19.18* | 23.80 | *19.43* | 47.8 |
| *18.57* | 23.03 | *18.79* | 23.32 | *19.04* | 23.63 | *19.29* | 23.93 | *19.54* | 48.0 |
| *18.67* | 23.16 | *18.90* | 23.45 | *19.14* | 23.76 | *19.40* | 24.06 | *19.65* | 48.2 |
| *18.77* | 23.28 | *19.00* | 23.58 | *19.25* | 23.89 | *19.50* | 24.19 | *19.76* | 48.4 |
| *18.88* | 23.41 | *19.11* | 23.71 | *19.36* | 24.02 | *19.61* | 24.32 | *19.87* | 48.6 |
| *18.98* | 23.54 | *19.21* | 23.83 | *19.47* | 24.14 | *19.72* | 24.45 | *19.98* | 48.8 |

22

## *Alcohol table.*—Continued.

| SCALE READING | 17.5°C. | | 18° C. | | 19° C. | | 20° C. | | 21° C. |
|---|---|---|---|---|---|---|---|---|---|
| | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume |
| 49.0 | 22.44 | 18.30 | 22.57 | 18.41 | 22.84 | 18.64 | 23.12 | 18.86 | 23.38 |
| 49.2 | 22.56 | 18.40 | 22.69 | 18.51 | 22.96 | 18.74 | 23.24 | 18.96 | 23.51 |
| 49.4 | 22.68 | 18.50 | 22.81 | 18.61 | 23.08 | 18.84 | 23.36 | 19.07 | 23.63 |
| 49.6 | 22.80 | 18.60 | 22.93 | 18.71 | 23.21 | 18.94 | 23.48 | 19.17 | 23.76 |
| 49.8 | 22.92 | 18.70 | 23.05 | 18.81 | 23.33 | 19.04 | 23.61 | 19.27 | 23.88 |
| 50.0 | 23.04 | 18.80 | 23.17 | 18.91 | 23.45 | 19.14 | 23.73 | 19.38 | 24.01 |
| 50.2 | 23.16 | 18.90 | 23.30 | 19.02 | 23.57 | 19.24 | 23.85 | 19.48 | 24.13 |
| 50.4 | 23.28 | 19.00 | 23.42 | 19.12 | 23.69 | 19.35 | 23.98 | 19.58 | 24.26 |
| 50.6 | 23.40 | 19.10 | 23.54 | 19.22 | 23.81 | 19.45 | 24.10 | 19.69 | 24.38 |
| 50.8 | 23.51 | 19.20 | 23.66 | 19.32 | 23.93 | 19.55 | 24.22 | 19.79 | 24.51 |
| 51.0 | 23.63 | 19.30 | 23.78 | 19.42 | 24.05 | 19.65 | 24.35 | 19.89 | 24.64 |
| 51.2 | 23.75 | 19.40 | 23.90 | 19.52 | 24.18 | 19.75 | 24.47 | 20.00 | 24.76 |
| 51.4 | 23.87 | 19.50 | 24.02 | 19.62 | 24.30 | 19.85 | 24.59 | 20.10 | 24.89 |
| 51.6 | 23.99 | 19.60 | 24.14 | 19.72 | 24.42 | 19.95 | 24.72 | 20.20 | 25.01 |
| 51.8 | 24.11 | 19.70 | 24.26 | 19.82 | 24.54 | 20.06 | 24.84 | 20.31 | 25.14 |
| 52.0 | 24.23 | 19.80 | 24.38 | 19.92 | 24.66 | 20.16 | 24.96 | 20.41 | 25.27 |
| 52.2 | 24.36 | 19.90 | 24.50 | 20.02 | 24.79 | 20.26 | 25.09 | 20.52 | 25.39 |
| 52.4 | 24.48 | 20.00 | 24.62 | 20.12 | 24.91 | 20.37 | 25.21 | 20.62 | 25.52 |
| 52.6 | 24.60 | 20.10 | 24.74 | 20.22 | 25.03 | 20.47 | 25.34 | 20.72 | 25.65 |
| 52.8 | 24.72 | 20.20 | 24.86 | 20.33 | 25.15 | 20.57 | 25.46 | 20.83 | 25.77 |
| 53.0 | 24.84 | 20.30 | 24.98 | 20.43 | 25.28 | 20.68 | 25.59 | 20.93 | 25.90 |
| 53.2 | 24.96 | 20.41 | 25.10 | 20.53 | 25.40 | 20.78 | 25.71 | 21.04 | 26.03 |
| 53.4 | 25.08 | 20.51 | 25.23 | 20.63 | 25.52 | 20.88 | 25.84 | 21.14 | 26.15 |
| 53.6 | 25.20 | 20.61 | 25.35 | 20.74 | 25.65 | 20.98 | 25.96 | 21.25 | 26.28 |
| 53.8 | 25.32 | 20.71 | 25.47 | 20.84 | 25.77 | 21.09 | 26.09 | 21.36 | 26.41 |
| 54.0 | 25.44 | 20.81 | 25.59 | 20.94 | 25.90 | 21.19 | 26.22 | 21.47 | 26.54 |
| 54.2 | 25.56 | 20.91 | 25.71 | 21.04 | 26.02 | 21.30 | 26.34 | 21.57 | 26.67 |
| 54.4 | 25.68 | 21.02 | 25.84 | 21.14 | 26.14 | 21.40 | 26.47 | 21.68 | 26.79 |
| 54.6 | 25.81 | 21.12 | 25.96 | 21.25 | 26.27 | 21.51 | 26.59 | 21.79 | 26.92 |
| 54.8 | 25.93 | 21.22 | 26.08 | 21.35 | 26.39 | 21.62 | 26.72 | 21.90 | 27.05 |
| 55.0 | 26.05 | 21.32 | 26.20 | 21.45 | 26.52 | 21.72 | 26.85 | 22.00 | 27.18 |
| 55.2 | 26.17 | 21.43 | 26.32 | 21.56 | 26.64 | 21.83 | 26.97 | 22.11 | 27.31 |
| 55.4 | 26.29 | 21.53 | 26.45 | 21.66 | 26.76 | 21.93 | 27.10 | 22.21 | 27.43 |
| 55.6 | 26.41 | 21.63 | 26.57 | 21.76 | 26.89 | 22.04 | 27.23 | 22.32 | 27.55 |
| 55.8 | 26.53 | 21.73 | 26.69 | 21.87 | 27.01 | 22.14 | 27.35 | 22.42 | 27.69 |
| 56.0 | 26.65 | 21.84 | 26.81 | 21.97 | 27.14 | 22.24 | 27.48 | 22.53 | 27.82 |
| 56.2 | 26.78 | 21.94 | 26.93 | 22.07 | 27.26 | 22.35 | 27.60 | 22.64 | 27.94 |
| 56.4 | 26.90 | 22.04 | 27.05 | 22.18 | 27.38 | 22.45 | 27.73 | 22.74 | 28.07 |
| 56.6 | 27.02 | 22.14 | 27.18 | 22.28 | 27.51 | 22.56 | 27.85 | 22.85 | 28.20 |
| 56.8 | 27.14 | 22.25 | 27.30 | 22.38 | 27.63 | 22.66 | 27.98 | 22.95 | 28.33 |
| 57.0 | 27.26 | 22.35 | 27.42 | 22.48 | 27.75 | 22.77 | 28.10 | 23.06 | 28.46 |
| 57.2 | 27.38 | 22.45 | 27.54 | 22.59 | 27.88 | 22.87 | 28.23 | 23.17 | 28.59 |
| 57.4 | 27.50 | 22.55 | 27.66 | 22.69 | 28.00 | 22.97 | 28.35 | 23.27 | 28.72 |
| 57.6 | 27.62 | 22.66 | 27.79 | 22.79 | 28.13 | 23.08 | 38.48 | 23.38 | 28.85 |
| 57.8 | 27.75 | 22.76 | 27.91 | 22.90 | 28.25 | 23.19 | 28.60 | 23.49 | 28.97 |

*Alcohol table.*—Continued.

| 21° C. | 22° C. | | 23° C. | | 24° C. | | 25° C. | | |
|---|---|---|---|---|---|---|---|---|---|
| Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | SCALE READING |
| 19.08 | 23.66 | 19.32 | 23.96 | 19.57 | 24.27 | 19.83 | 24.59 | 20.09 | 49.0 |
| 19.19 | 23.79 | 19.43 | 24.09 | 19.68 | 24.40 | 19.94 | 24.72 | 20.20 | 49.2 |
| 19.29 | 23.92 | 19.54 | 24.22 | 19.79 | 24.53 | 20.04 | 24.85 | 20.31 | 49.4 |
| 19.40 | 24.04 | 19.64 | 24.35 | 19.89 | 24.66 | 20.15 | 24.98 | 20.42 | 49.6 |
| 19.51 | 24.17 | 19.75 | 24.48 | 20.00 | 24.79 | 20.27 | 25.11 | 20.54 | 49.8 |
| 19.61 | 24.30 | 19.86 | 24.61 | 20.11 | 24.92 | 20.38 | 25.25 | 20.65 | 50.0 |
| 19.72 | 24.43 | 19.96 | 24.74 | 20.22 | 25.05 | 20.49 | 25.38 | 20.76 | 50.2 |
| 19.82 | 24.56 | 20.07 | 24.86 | 20.33 | 25.18 | 20.60 | 25.51 | 20.87 | 50.4 |
| 19.93 | 24.69 | 20.18 | 24.99 | 20.44 | 25.32 | 20.71 | 25.65 | 20.98 | 50.6 |
| 20.03 | 24.81 | 20.29 | 25.12 | 20.55 | 25.45 | 20.82 | 25.78 | 21.10 | 50.8 |
| 20.14 | 24.94 | 20.39 | 25.25 | 20.66 | 25.58 | 20.93 | 25.91 | 21.21 | 51.0 |
| 20.24 | 25.07 | 20.50 | 25.38 | 20.77 | 25.71 | 21.04 | 26.05 | 21.32 | 51.2 |
| 20.35 | 25.20 | 20.61 | 25.51 | 20.87 | 25.84 | 21.15 | 26.18 | 21.44 | 51.4 |
| 20.46 | 25.33 | 20.72 | 25.64 | 20.98 | 25.97 | 21.26 | 26.32 | 21.55 | 51.6 |
| 20.56 | 25.46 | 20.82 | 25.77 | 21.09 | 26.11 | 21.37 | 26.45 | 21.66 | 51.8 |
| 20.67 | 25.58 | 20.93 | 25.90 | 21.20 | 26.24 | 21.49 | 26.59 | 21.78 | 52.0 |
| 20.77 | 25.71 | 21.04 | 26.03 | 21.31 | 26.37 | 21.60 | 26.72 | 21.89 | 52.2 |
| 20.88 | 25.84 | 21.15 | 26.16 | 21.42 | 26.51 | 21.71 | 26.86 | 22.01 | 52.4 |
| 20.98 | 25.97 | 21.26 | 26.29 | 21.53 | 26.64 | 21.82 | 26.99 | 22.12 | 52.6 |
| 21.09 | 26.10 | 21.36 | 26.42 | 21.64 | 26.77 | 21.93 | 27.13 | 22.24 | 52.8 |
| 21.20 | 26.23 | 21.47 | 26.56 | 21.75 | 26.91 | 22.05 | 27.27 | 22.35 | 53.0 |
| 21.31 | 26.35 | 21.58 | 26.69 | 21.86 | 27.04 | 22.16 | 27.40 | 22.47 | 53.2 |
| 21.42 | 26.48 | 21.69 | 26.82 | 21.97 | 27.17 | 22.27 | 27.54 | 22.58 | 53.4 |
| 21.52 | 26.61 | 21.80 | 26.95 | 22.08 | 27.31 | 22.39 | 27.67 | 22.70 | 53.6 |
| 21.63 | 26.74 | 21.91 | 27.08 | 22.20 | 27.44 | 22.50 | 27.81 | 22.81 | 53.8 |
| 21.74 | 26.87 | 22.02 | 27.21 | 22.31 | 27.58 | 22.61 | 27.95 | 22.93 | 54.0 |
| 21.85 | 27.00 | 22.13 | 27.35 | 22.42 | 27.71 | 22.73 | 28.08 | 23.04 | 54.2 |
| 21.96 | 27.13 | 22.24 | 27.48 | 22.53 | 27.85 | 22.84 | 28.22 | 23.16 | 54.4 |
| 22.06 | 27.26 | 22.35 | 27.61 | 22.65 | 27.98 | 22.95 | 28.36 | 23.27 | 54.6 |
| 22.17 | 27.39 | 22.46 | 27.75 | 22.76 | 28.11 | 23.07 | 28.49 | 23.39 | 54.8 |
| 22.28 | 27.52 | 22.57 | 27.88 | 22.87 | 28.25 | 23.18 | 28.63 | 23.51 | 55.0 |
| 22.39 | 27.65 | 22.68 | 28.01 | 22.98 | 28.38 | 23.30 | 28.77 | 23.62 | 55.2 |
| 22.49 | 27.78 | 22.79 | 28.15 | 23.10 | 28.52 | 23.41 | 28.90 | 23.74 | 55.4 |
| 22.60 | 27.92 | 22.90 | 28.28 | 23.21 | 28.65 | 23.53 | 29.04 | 23.86 | 55.6 |
| 22.71 | 28.05 | 23.01 | 28.41 | 23.32 | 28.78 | 23.64 | 29.18 | 23.97 | 55.8 |
| 22.82 | 28.18 | 23.12 | 28.54 | 23.43 | 28.92 | 23.75 | 29.31 | 24.09 | 56.0 |
| 22.92 | 28.31 | 23.23 | 28.68 | 23.54 | 29.05 | 23.87 | 29.45 | 24.20 | 56.2 |
| 23.03 | 28.44 | 23.34 | 28.81 | 23.66 | 29.19 | 23.98 | 29.58 | 24.32 | 56.4 |
| 23.14 | 28.56 | 23.45 | 28.94 | 23.77 | 29.32 | 24.10 | 29.72 | 24.43 | 56.6 |
| 23.25 | 28.69 | 23.56 | 29.07 | 23.88 | 29.46 | 24.21 | 28.86 | 24.55 | 56.8 |
| 23.36 | 28.82 | 23.67 | 29.20 | 23.99 | 29.59 | 24.32 | 29.99 | 24.66 | 57.0 |
| 23.47 | 28.95 | 23.78 | 29.34 | 24.11 | 29.73 | 24.44 | 30.13 | 24.78 | 57.2 |
| 23.58 | 29.08 | 23.90 | 29.47 | 24.22 | 29.86 | 24.55 | 30.27 | 24.90 | 57.4 |
| 23.69 | 29.21 | 24.01 | 29.60 | 24.33 | 30.00 | 24.66 | 30.41 | 25.01 | 57.6 |
| 23.80 | 29.34 | 24.12 | 29.73 | 24.44 | 30.14 | 24.78 | 30.55 | 25.13 | 57.8 |

22 *Alcohol table.*—Continued.

| SCALE READING | 17.5° C. | | 18° C. | | 19° C. | | 20° C. | | 21° C. |
|---|---|---|---|---|---|---|---|---|---|
| | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume |
| 58.0 | 27.87 | *22.86* | 28.03 | *23.00* | 28.38 | *23.29* | 28.73 | *23.59* | 29.10 |
| 58.2 | 27.99 | *22.96* | 28.15 | *23.10* | 28.50 | *23.40* | 28.86 | *23.70* | 29.23 |
| 58.4 | 28.11 | *23.07* | 28.28 | *23.21* | 28.62 | *23.50* | 28.98 | *23.81* | 29.36 |
| 58.6 | 28.23 | *23.17* | 28.40 | *23.31* | 28.75 | *23.61* | 29.11 | *23.91* | 29.48 |
| 58.8 | 28.35 | *23.27* | 28.52 | *23.41* | 28.88 | *23.71* | 29.23 | *24.02* | 29.61 |
| 59.0 | 28.47 | *23.37* | 28.64 | *23.52* | 29.00 | *23.82* | 29.36 | *24.13* | 29.74 |
| 59.2 | 28.59 | *23.48* | 28.77 | *23.62* | 29.12 | *23.93* | 29.49 | *24.24* | 29.87 |
| 59.4 | 28.71 | *23.58* | 28.89 | *23.73* | 29.25 | *24.03* | 29.61 | *24.34* | 29.99 |
| 59.6 | 28.84 | *23.68* | 29.01 | *23.83* | 29.37 | *24.14* | 29.74 | *24.45* | 30.13 |
| 59.8 | 28.96 | *23.79* | 29.13 | *23.93* | 29.50 | *24.24* | 28.87 | *24.56* | 30.26 |
| 60.0 | 29.08 | *23.89* | 29.26 | *24.04* | 29.62 | *24.35* | 29.99 | *24.67* | 30.39 |
| 60.2 | 29.20 | *23.99* | 29.38 | *24.14* | 29.74 | *24.46* | 30.12 | *24.77* | 30.52 |
| 60.4 | 29.32 | *24.10* | 29.50 | *24.25* | 28.87 | *24.56* | 30.25 | *24.88* | 30.65 |
| 60.6 | 29.45 | *24.20* | 29.63 | *24.35* | 29.99 | *24.67* | 30.38 | *24.99* | 30.78 |
| 60.8 | 29.57 | *24.30* | 29.75 | *24.46* | 30.12 | *24.77* | 30.51 | *25.10* | 30.91 |
| 61.0 | 29.69 | *24.41* | 29.87 | *24.56* | 30.25 | *24.88* | 30.64 | *25.21* | 31.05 |
| 61.2 | 29.81 | *24.51* | 29.99 | *24.66* | 30.38 | *24.98* | 30.77 | *25.32* | 31.18 |
| 61.4 | 29.93 | *24.61* | 30.12 | *24.77* | 30.50 | *25.09* | 30.90 | *25.44* | 31.32 |
| 61.6 | 30.06 | *24.72* | 30.25 | *24.87* | 30.63 | *25.20* | 31.03 | *25.55* | 31.45 |
| 61.8 | 30.18 | *24.82* | 30.37 | *25.98* | 30.76 | *25.31* | 31.16 | *25.66* | 31.59 |
| 62.0 | 30.31 | *24.93* | 30.50 | *25.09* | 30.89 | *25.43* | 31.29 | *25.77* | 31.72 |
| 62.2 | 30.43 | *25.03* | 30.63 | *25.20* | 31.01 | *25.54* | 31.43 | *25.88* | 31.86 |
| 62.4 | 30.56 | *25.14* | 30.75 | *25.31* | 31.14 | *25.65* | 31.56 | *25.99* | 31.99 |
| 62.6 | 30.69 | *25.25* | 30.88 | *25.42* | 31.28 | *25.76* | 31.69 | *26.11* | 32.13 |
| 62.8 | 30.81 | *25.36* | 31.01 | *25.53* | 31.41 | *25.87* | 31.83 | *26.23* | 32.27 |
| 63.0 | 30.94 | *25.47* | 31.14 | *25.64* | 31.54 | *25.98* | 31.96 | *26.35* | 32.41 |
| 63.2 | 31.06 | *25.58* | 31.26 | *25.75* | 31.67 | *26.09* | 32.10 | *26.46* | 32.55 |
| 63.4 | 31.19 | *25.69* | 31.39 | *25.86* | 31.80 | *26.21* | 32.23 | *26.58* | 32.69 |
| 63.6 | 31.32 | *25.80* | 31.52 | *25.97* | 31.93 | *26.32* | 32.37 | *26.70* | 32.83 |
| 63.8 | 31.45 | *25.91* | 31.65 | *26.08* | 32.07 | *26.44* | 32.51 | *26.82* | 32.97 |
| 64.0 | 31.58 | *26.02* | 31.78 | *26.19* | 32.20 | *26.55* | 32.65 | *26.94* | 33.11 |
| 64.2 | 31.70 | *26.13* | 31.91 | *26.30* | 32.34 | *26.67* | 32.79 | *27.05* | 33.25 |
| 64.4 | 31.83 | *26.24* | 32.04 | *26.41* | 32.47 | *26.78* | 32.92 | *27.17* | 33.39 |
| 64.6 | 31.96 | *26.35* | 32.17 | *26.53* | 32.60 | *26.90* | 33.06 | *27.29* | 33.53 |
| 64.8 | 32.09 | *26.46* | 32.30 | *26.64* | 32.74 | *27.01* | 33.20 | *27.41* | 33.67 |
| 65.0 | 32.22 | *26.57* | 32.43 | *26.75* | 32.87 | *27.13* | 33.34 | *27.53* | 33.82 |
| 65.2 | 32.35 | *26.68* | 32.57 | *26.86* | 33.01 | *27.25* | 33.48 | *27.65* | 33.96 |
| 65.4 | 32.48 | *26.79* | 32.70 | *26.97* | 33.15 | *27.37* | 33.62 | *27.77* | 34.10 |
| 65.6 | 32.61 | *26.90* | 32.83 | *27.09* | 33.28 | *27.49* | 33.76 | *27.89* | 34.25 |
| 65.8 | 32.75 | *27.01* | 32.96 | *27.21* | 33.42 | *27.60* | 33.90 | *28.01* | 34.40 |
| 66.0 | 32.88 | *27.13* | 33.10 | *27.32* | 33.56 | *27.72* | 34.04 | *28.13* | 34.54 |
| 66.2 | 33.01 | *27.25* | 33.23 | *27.44* | 33.70 | *27.84* | 34.18 | *28.26* | 34.69 |
| 66.4 | 33.14 | *27.36* | 33.37 | *27.56* | 33.84 | *27.96* | 34.33 | *28.38* | 34.84 |
| 66.6 | 33.28 | *27.48* | 33.51 | *27.68* | 33.98 | *28.08* | 34.47 | *28.51* | 34.99 |
| 66.8 | 33.41 | *27.60* | 33.65 | *27.79* | 34.12 | *28.20* | 34.62 | *28.64* | 35.14 |

*Alcohol table.*—Continued.

| 21° C. | 22° C. | | 23° C. | | 24° C. | | 25° C. | | SCALE READING |
|---|---|---|---|---|---|---|---|---|---|
| Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | |
| 23.91 | 29.47 | 24.23 | 29.87 | 24.55 | 30.27 | 24.89 | 30.69 | 25.25 | 58.0 |
| 24.02 | 29.60 | 24.34 | 29.99 | 24.66 | 30.41 | 25.01 | 30.83 | 25.37 | 58.2 |
| 24.13 | 29.73 | 24.45 | 30.13 | 24.78 | 30.54 | 25.13 | 30.97 | 25.49 | 58.4 |
| 24.23 | 29.86 | 24.56 | 30.26 | 24.89 | 30.68 | 25.23 | 31.11 | 25.61 | 58.6 |
| 24.34 | 29.99 | 24.67 | 30.40 | 25.00 | 30.82 | 25.35 | 31.25 | 25.73 | 58.8 |
| 24.45 | 30.13 | 24.78 | 30.53 | 25.12 | 30.95 | 25.47 | 31.40 | 25.86 | 59.0 |
| 24.56 | 30.26 | 24.89 | 30.67 | 25.24 | 31.09 | 25.59 | 31.54 | 25.98 | 59.2 |
| 24.67 | 30.39 | 25.00 | 30.81 | 25.36 | 31.23 | 25.71 | 31.68 | 26.10 | 59.4 |
| 24.78 | 30.53 | 25.11 | 30.94 | 25.47 | 31.38 | 25.83 | 31.83 | 26.23 | 59.6 |
| 24.89 | 30.66 | 25.23 | 31.08 | 25.59 | 31.52 | 25.95 | 31.97 | 26.35 | 59.8 |
| 24.99 | 30.79 | 25.34 | 31.22 | 25.71 | 31.66 | 26.08 | 32.12 | 26.48 | 60.0 |
| 25.11 | 30.93 | 25.46 | 31.36 | 25.83 | 31.80 | 26.20 | 32.27 | 26.61 | 60.2 |
| 25.22 | 31.06 | 25.57 | 31.50 | 25.95 | 31.94 | 26.33 | 23.41 | 26.73 | 60.4 |
| 25.34 | 31.20 | 25.69 | 31.64 | 26.07 | 32.09 | 26.45 | 32.56 | 27.86 | 60.6 |
| 25.45 | 31.33 | 25.80 | 31.78 | 26.19 | 32.23 | 26.58 | 32.71 | 26.99 | 60.8 |
| 25.56 | 31.47 | 25.92 | 31.92 | 26.31 | 32.38 | 26.70 | 32.86 | 27.12 | 61.0 |
| 25.68 | 31.61 | 26.04 | 32.06 | 26.43 | 32.52 | 26.83 | 33.01 | 27.24 | 61.2 |
| 25.79 | 31.74 | 26.16 | 32.20 | 26.55 | 32.67 | 26.95 | 33.16 | 27.37 | 61.4 |
| 25.90 | 31.88 | 26.28 | 32.34 | 26.67 | 32.81 | 27.08 | 33.31 | 27.50 | 61.6 |
| 26.02 | 32.01 | 26.40 | 32.49 | 26.79 | 32.96 | 27.20 | 33.46 | 27.63 | 61.8 |
| 26.14 | 32.16 | 26.51 | 32.63 | 26.92 | 33.10 | 27.33 | 33.60 | 27.76 | 62.0 |
| 26.25 | 32.30 | 26.63 | 32.77 | 27.04 | 33.25 | 27.46 | 33.75 | 27.88 | 62.2 |
| 26.37 | 32.44 | 26.75 | 32.91 | 27.16 | 33.40 | 27.58 | 33.90 | 28.01 | 62.6 |
| 26.49 | 32.58 | 26.87 | 33.06 | 27.29 | 33.55 | 27.71 | 34.05 | 28.15 | 62.6 |
| 26.61 | 32.72 | 26.99 | 33.20 | 27.41 | 33.70 | 27.84 | 34.21 | 28.28 | 62.8 |
| 26.73 | 32.87 | 27.12 | 33.35 | 27.54 | 33.84 | 27.96 | 34.36 | 28.42 | 63.0 |
| 26.85 | 33.01 | 27.24 | 33.50 | 27.66 | 33.99 | 28.09 | 34.52 | 28.55 | 63.2 |
| 26.96 | 33.15 | 27.37 | 33.64 | 27.79 | 34.15 | 28.23 | 34.67 | 28.69 | 63.4 |
| 27.09 | 33.30 | 27.49 | 33.79 | 27.91 | 34.30 | 28.36 | 34.83 | 28.82 | 63.6 |
| 27.21 | 33.44 | 27.62 | 33.93 | 28.04 | 34.45 | 28.49 | 34.98 | 28.96 | 63.8 |
| 27.33 | 33.59 | 27.74 | 34.08 | 28.17 | 34.61 | 28.63 | 35.15 | 29.10 | 64.0 |
| 27.45 | 33.73 | 27.87 | 34.23 | 28.30 | 34.76 | 28.76 | 35.31 | 29.24 | 64.2 |
| 27.57 | 33.88 | 27.99 | 34.39 | 28.43 | 34.92 | 28.89 | 35.48 | 29.38 | 64.4 |
| 27.70 | 34.02 | 28.12 | 34.54 | 28.57 | 35.07 | 29.03 | 35.64 | 29.52 | 64.6 |
| 27.82 | 34.17 | 28.25 | 34.69 | 28.70 | 35.23 | 29.17 | 35.80 | 29.67 | 64.8 |
| 27.94 | 34.32 | 28.38 | 34.84 | 28.83 | 35.39 | 29.31 | 35.97 | 29.81 | 65.0 |
| 28.06 | 34.47 | 28.51 | 34.99 | 28.96 | 35.55 | 29.44 | 36.13 | 29.95 | 65.2 |
| 28.19 | 34.61 | 28.63 | 35.15 | 29.10 | 35.71 | 29.58 | 36.30 | 30.10 | 65.4 |
| 28.32 | 34.76 | 28.76 | 35.30 | 29.23 | 35.87 | 29.72 | 36.46 | 30.24 | 65.6 |
| 28.45 | 34.91 | 28.89 | 35.46 | 29.37 | 36.02 | 29.86 | 36.63 | 30.39 | 65.8 |
| 28.57 | 35.06 | 29.02 | 35.62 | 29.51 | 36.19 | 30.00 | 36.79 | 30.54 | 66.0 |
| 28.70 | 35.22 | 29.16 | 35.77 | 29.64 | 36.35 | 30.15 | 36.96 | 30.68 | 66.2 |
| 28.83 | 35.38 | 29.29 | 35.93 | 29.78 | 36.52 | 30.29 | 37.13 | 30.83 | 66.4 |
| 28.96 | 35.53 | 29.43 | 36.09 | 29.92 | 36.68 | 30.43 | 37.30 | 30.98 | 66.6 |
| 29.09 | 35.69 | 29.57 | 36.25 | 30.06 | 36.84 | 30.58 | 37.48 | 31.13 | 66.8 |

22 *Alcohol table.*—Continued.

| SCALE READING | 17.5° C. | | 18° C. | | 19° C. | | 20° C. | | 21° C. |
|---|---|---|---|---|---|---|---|---|---|
| | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume |
| 67.0 | 33.55 | 27.71 | 33.79 | 27.91 | 34.26 | 28.33 | 34.76 | 28.76 | 35.29 |
| 67.2 | 33.69 | 27.83 | 33.92 | 28.03 | 34.41 | 28.45 | 34.91 | 28.89 | 35.44 |
| 67.4 | 33.82 | 27.95 | 34.06 | 27.15 | 34.55 | 28.58 | 35.05 | 29.01 | 35.60 |
| 67.6 | 33.96 | 28.06 | 34.20 | 28.27 | 34.69 | 28.70 | 35.20 | 29.14 | 35.75 |
| 67.8 | 34.09 | 28.18 | 34.34 | 28.40 | 34.84 | 28.83 | 35.35 | 29.27 | 35.90 |
| 68.0 | 34.23 | 28.30 | 34.48 | 28.52 | 34.98 | 28.95 | 35.50 | 29.41 | 36.05 |
| 68.2 | 34.36 | 28.42 | 34.62 | 28.64 | 35.13 | 29.08 | 35.65 | 29.54 | 36.21 |
| 68.4 | 34.50 | 28.54 | 34.76 | 28.76 | 35.27 | 29.21 | 35.80 | 29.67 | 36.37 |
| 68.6 | 34.64 | 28.65 | 34.90 | 28.88 | 35.42 | 29.33 | 35.95 | 29.80 | 36.52 |
| 68.8 | 34.77 | 28.77 | 35.04 | 29.01 | 35.57 | 29.46 | 36.10 | 29.93 | 36.68 |
| 69.0 | 34.91 | 28.89 | 35.19 | 29.13 | 35.71 | 29.59 | 36.25 | 30.06 | 36.84 |
| 69.2 | 35.04 | 29.01 | 35.33 | 29.26 | 35.86 | 29.72 | 36.41 | 30.20 | 36.99 |
| 69.4 | 35.19 | 29.14 | 35.47 | 29.38 | 36.01 | 29.85 | 36.56 | 30.33 | 37.15 |
| 69.6 | 35.34 | 29.26 | 35.62 | 29.51 | 36.16 | 29.97 | 36.72 | 30.47 | 37.32 |
| 69.8 | 35.49 | 29.39 | 35.76 | 29.64 | 36.31 | 30.11 | 36.87 | 30.61 | 37.48 |
| 70.0 | 35.64 | 29.52 | 35.91 | 29.76 | 36.46 | 30.24 | 37.02 | 30.74 | 37.64 |
| 70.2 | 35.78 | 29.65 | 36.05 | 29.89 | 36.61 | 30.38 | 37.19 | 30.88 | 37.80 |
| 70.4 | 35.93 | 29.78 | 36.20 | 30.01 | 36.76 | 30.51 | 37.35 | 31.01 | 37.97 |
| 70.6 | 36.08 | 29.91 | 36.35 | 30.15 | 36.92 | 30.64 | 37.51 | 31.16 | 38.13 |
| 70.8 | 36.23 | 30.04 | 36.50 | 30.28 | 37.07 | 30.78 | 37.67 | 31.30 | 38.30 |
| 71.0 | 36.38 | 30.17 | 36.65 | 30.41 | 37.23 | 30.91 | 37.83 | 31.44 | 38.47 |
| 71.2 | 36.53 | 30.30 | 36.80 | 30.55 | 37.39 | 31.05 | 37.99 | 31.59 | 38.63 |
| 71.4 | 36.68 | 30.44 | 36.95 | 30.68 | 37.55 | 31.19 | 38.16 | 31.73 | 38.80 |
| 71.6 | 36.83 | 30.57 | 37.11 | 30.81 | 37.71 | 31.33 | 38.32 | 31.87 | 38.97 |
| 71.8 | 36.98 | 30.70 | 37.27 | 30.95 | 37.87 | 31.47 | 38.49 | 32.01 | 39.14 |
| 72.0 | 37.13 | 30.84 | 37.42 | 31.08 | 38.02 | 31.61 | 38.65 | 32.17 | 39.31 |
| 72.2 | 37.29 | 30.97 | 37.58 | 31.22 | 38.19 | 31.75 | 38.82 | 32.32 | 39.49 |
| 72.4 | 37.44 | 31.10 | 37.73 | 31.36 | 38.35 | 31.89 | 38.98 | 32.47 | 39.66 |
| 72.6 | 37.60 | 31.24 | 37.89 | 31.49 | 38.51 | 32.04 | 39.16 | 32.62 | 39.83 |
| 72.8 | 37.75 | 31.37 | 38.05 | 31.63 | 38.67 | 32.18 | 39.33 | 32.77 | 40.01 |
| 73.0 | 37.91 | 31.51 | 38.21 | 31.77 | 38.84 | 32.33 | 39.50 | 32.92 | 40.18 |
| 73.2 | 38.06 | 31.65 | 38.37 | 31.90 | 39.00 | 32.48 | 39.67 | 33.07 | 40.36 |
| 73.4 | 38.22 | 31.78 | 38.53 | 32.04 | 39.17 | 32.62 | 39.84 | 33.22 | 40.53 |
| 73.6 | 38.38 | 31.92 | 38.69 | 32.19 | 39.34 | 32.77 | 40.02 | 33.37 | 40.70 |
| 73.8 | 38.54 | 32.06 | 38.85 | 32.34 | 39.50 | 32.92 | 40.19 | 33.53 | 40.88 |
| 74.0 | 38.70 | 32.20 | 39.01 | 32.48 | 39.67 | 33.07 | 40.36 | 33.68 | 41.05 |
| 74.2 | 38.86 | 32.35 | 39.18 | 32.63 | 39.84 | 33.22 | 40.53 | 33.83 | 41.23 |
| 74.4 | 39.02 | 32.49 | 39.34 | 32.77 | 40.01 | 33.37 | 40.71 | 33.98 | 41.41 |
| 74.6 | 39.18 | 32.63 | 39.51 | 32.92 | 40.18 | 33.53 | 40.88 | 34.14 | 41.59 |
| 74.8 | 39.35 | 32.78 | 39.68 | 33.07 | 40.35 | 33.68 | 41.05 | 34.30 | 41.77 |
| 75.0 | 39.51 | 32.92 | 39.84 | 33.22 | 40.53 | 33.83 | 41.23 | 34.46 | 41.95 |
| 75.2 | 39.68 | 33.07 | 40.01 | 33.37 | 40.70 | 33.98 | 41.41 | 34.61 | 42.13 |
| 75.4 | 39.84 | 33.22 | 40.18 | 33.53 | 40.87 | 34.14 | 41.58 | 34.77 | 42.31 |
| 75.6 | 40.01 | 33.37 | 40.35 | 33.68 | 41.04 | 34.30 | 41.76 | 34.93 | 42.49 |
| 75.8 | 40.18 | 33.53 | 40.53 | 33.83 | 41.22 | 34.45 | 41.94 | 35.09 | 42.67 |

## *Alcohol table.*—Continued. 22

| 21° C. | 22° C. | | 23° C. | | 24° C. | | 25° C. | | |
|---|---|---|---|---|---|---|---|---|---|
| Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | SCALE READING |
| 29.22 | 35.84 | 29.71 | 36.41 | 30.20 | 37.01 | 30.72 | 37.65 | 31.28 | 67.0 |
| 29.35 | 36.00 | 29.84 | 36.57 | 30.34 | 37.18 | 30.87 | 37.83 | 31.44 | 67.2 |
| 29.49 | 36.16 | 29.98 | 36.73 | 30.49 | 37.35 | 31.02 | 38.00 | 31.59 | 67.4 |
| 29.62 | 36.32 | 30.12 | 36.90 | 30.63 | 37.52 | 31.17 | 38.18 | 31.74 | 67.6 |
| 29.75 | 36.48 | 30.26 | 37.06 | 30.77 | 37.69 | 31.32 | 38.35 | 31.89 | 67.8 |
| 29.89 | 36.63 | 30.40 | 37.23 | 30.91 | 37.86 | 31.47 | 38.53 | 32.05 | 68.0 |
| 30.02 | 36.79 | 30.54 | 37.39 | 31.06 | 38.03 | 31.62 | 38.70 | 32.21 | 68.2 |
| 30.16 | 36.95 | 30.68 | 37.56 | 31.21 | 38.21 | 31.77 | 38.88 | 32.37 | 68.4 |
| 30.30 | 37.12 | 30.82 | 37.73 | 31.35 | 38.38 | 31.92 | 39.06 | 32.53 | 68.6 |
| 30.43 | 37.28 | 30.96 | 37.90 | 31.50 | 38.56 | 32.07 | 39.24 | 32.69 | 68.8 |
| 30.57 | 37.45 | 31.10 | 38.07 | 31.65 | 38.73 | 32.23 | 39.43 | 32.86 | 69.0 |
| 30.71 | 37.61 | 31.25 | 38.24 | 31.79 | 38.90 | 32.39 | 39.61 | 33.02 | 69.2 |
| 30.85 | 37.78 | 31.39 | 38.41 | 31.94 | 39.08 | 32.55 | 39.80 | 33.18 | 69.4 |
| 30.99 | 37.94 | 31.54 | 38.58 | 32.09 | 39.26 | 32.71 | 39.98 | 33.34 | 69.6 |
| 31.13 | 38.11 | 31.68 | 38.75 | 32.25 | 39.45 | 32.86 | 40.17 | 33.51 | 69.8 |
| 31.27 | 38.28 | 31.83 | 38.92 | 32.41 | 39.63 | 33.02 | 40.35 | 33.67 | 70.0 |
| 31.42 | 38.45 | 31.97 | 39.10 | 32.57 | 39.81 | 33.19 | 40.53 | 33.84 | 70.2 |
| 31.56 | 38.61 | 32.12 | 39.28 | 32.72 | 39.99 | 33.35 | 40.72 | 34.00 | 70.4 |
| 31.70 | 38.78 | 32.28 | 39.46 | 32.88 | 40.17 | 23.51 | 40.90 | 34.17 | 70.6 |
| 31.85 | 38.95 | 32.43 | 39.64 | 33.04 | 40.35 | 33.68 | 41.08 | 34.33 | 70.7 |
| 31.99 | 39.12 | 32.59 | 39.82 | 33.20 | 40.54 | 33.84 | 41.27 | 34.50 | 71.0 |
| 32.15 | 39.30 | 32.74 | 40.00 | 33.36 | 40.72 | 34.00 | 41.46 | 34.67 | 71.2 |
| 32.30 | 39.48 | 32.90 | 40.18 | 33.52 | 40.90 | 34.17 | 41.64 | 34.83 | 71.4 |
| 32.45 | 39.65 | 33.05 | 40.36 | 33.68 | 41.08 | 34.33 | 41.83 | 35.00 | 71.6 |
| 32.60 | 39.83 | 33.21 | 40.54 | 33.84 | 41.27 | 34.50 | 42.02 | 35.17 | 71.8 |
| 32.76 | 40.01 | 33.37 | 40.72 | 34.00 | 41.45 | 34.66 | 42.21 | 35.34 | 72.0 |
| 32.91 | 40.18 | 33.52 | 40.90 | 34.16 | 41.64 | 34.83 | 42.40 | 35.51 | 72.2 |
| 33.06 | 40.36 | 33.68 | 41.08 | 34.33 | 41.82 | 34.99 | 42.58 | 35.68 | 72.4 |
| 33.22 | 40.54 | 33.84 | 41.26 | 34.49 | 42.01 | 35.16 | 42.77 | 35.85 | 72.6 |
| 33.37 | 40.71 | 33.99 | 41.45 | 34.65 | 42.19 | 35.33 | 42.96 | 36.02 | 72.8 |
| 33.52 | 40.88 | 34.15 | 41.63 | 34.81 | 42.38 | 35.49 | 43.15 | 36.18 | 73.0 |
| 33.68 | 41.06 | 34.31 | 41.81 | 34.98 | 42.56 | 35.66 | 43.33 | 36.35 | 73.2 |
| 33.83 | 41.24 | 34.47 | 41.99 | 35.14 | 42.75 | 35.83 | 43.52 | 36.52 | 73.4 |
| 33.98 | 41.42 | 34.63 | 42.17 | 35.31 | 42.93 | 35.99 | 43.70 | 36.68 | 73.6 |
| 34.14 | 41.60 | 34.79 | 42.36 | 35.47 | 43.12 | 36.16 | 43.89 | 36.85 | 73.8 |
| 34.30 | 41.78 | 34.95 | 42.54 | 35.64 | 43.31 | 36.33 | 44.08 | 37.02 | 74.0 |
| 34.46 | 41.96 | 35.12 | 42.72 | 35.80 | 43.49 | 36.49 | 44.28 | 37.20 | 74.2 |
| 34.62 | 42.15 | 35.28 | 42.91 | 35.97 | 43.68 | 36.66 | 44.48 | 37.38 | 74.4 |
| 34.78 | 42.33 | 35.45 | 43.09 | 36.13 | 43.86 | 36.83 | 44.67 | 37.56 | 74.6 |
| 34.94 | 42.51 | 35.61 | 43.28 | 36.30 | 44.05 | 36.99 | 44.87 | 37.75 | 74.8 |
| 35.10 | 42.70 | 35.78 | 43.46 | 36.47 | 44.25 | 37.17 | 45.07 | 37.93 | 75.0 |
| 35.26 | 42.88 | 35.95 | 43.65 | 36.64 | 44.44 | 37.35 | 45.29 | 38.12 | 75.2 |
| 35.43 | 43.07 | 36.11 | 43.83 | 36.81 | 44.63 | 37.53 | 45.50 | 38.31 | 75.4 |
| 35.59 | 43.25 | 36.28 | 44.02 | 36.97 | 44.83 | 37.71 | 45.71 | 38.50 | 75.6 |
| 35.75 | 43.44 | 36.45 | 44.21 | 37.15 | 45.03 | 37.89 | 45.92 | 38.69 | 75.8 |

22 *Alcohol table.*—Continued.

| SCALE READING | 17.5° C. | | 18° C. | | 19° C. | | 20° C. | | 21° C. |
|---|---|---|---|---|---|---|---|---|---|
| | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume |
| 76.0 | 40.35 | *33.68* | 40.70 | *33.98* | 41.40 | *34.61* | 42.12 | *35.25* | 42.85 |
| 76.2 | 40.53 | *33.83* | 40.87 | *34.14* | 41.57 | *34.77* | 42.30 | *35.41* | 43.04 |
| 76.4 | 40.70 | *33.98* | 41.04 | *34.29* | 41.75 | *34.92* | 42.48 | *35.58* | 43.22 |
| 76.6 | 40.87 | *34.14* | 41.22 | *34.45* | 41.92 | *35.08* | 42.66 | *35.74* | 43.41 |
| 76.8 | 41.04 | *34.29* | 41.39 | *34.60* | 42.10 | *35.24* | 42.84 | *35.90* | 43.60 |
| 77.0 | 41.22 | *34.45* | 41.57 | *34.76* | 42.28 | *35.40* | 43.02 | *36.07* | 43.79 |
| 77.2 | 41.39 | *34.60* | 41.74 | *34.91* | 42.46 | *35.56* | 43.20 | *36.24* | 43.97 |
| 77.4 | 41.57 | *34.75* | 41.91 | *35.07* | 42.63 | *35.72* | 43.39 | *36.40* | 44.16 |
| 77.6 | 41.75 | *34.91* | 42.09 | *35.23* | 42.81 | *35.88* | 43.57 | *36.57* | 44.35 |
| 77.8 | 41.92 | *35.06* | 42.26 | *35.38* | 42.99 | *36.04* | 43.76 | *36.74* | 44.54 |
| 78.0 | 42.09 | *35.22* | 42.43 | *35.54* | 43.17 | *36.21* | 43.94 | *36.91* | 44.73 |
| 78.2 | 42.26 | *35.38* | 42.61 | *35.70* | 43.36 | *36.38* | 44.13 | *37.08* | 44.92 |
| 78.4 | 42.44 | *35.53* | 42.78 | *35.85* | 43.54 | *36.54* | 44.32 | *37.25* | 45.12 |
| 78.6 | 42.61 | *35.69* | 42.96 | *36.01* | 43.72 | *36.71* | 44.51 | *37.42* | 45.32 |
| 78.8 | 42.78 | *35.84* | 43.14 | *36.17* | 43.91 | *36.88* | 44.70 | *37.59* | 45.52 |
| 79.0 | 42.95 | *36.00* | 43.32 | *36.34* | 44.09 | *37.04* | 44.89 | *37.76* | 45.72 |
| 79.2 | 43.13 | *36.16* | 43.50 | *36.50* | 44.28 | *37.21* | 45.08 | *37.94* | 45.92 |
| 79.4 | 43.31 | *36.33* | 43.68 | *36.67* | 44.47 | *37.38* | 45.28 | *38.11* | 46.13 |
| 79.6 | 43.49 | *36.49* | 43.86 | *36.83* | 44.65 | *37.56* | 45.48 | *38.30* | 46.34 |
| 79.8 | 43.67 | *36.66* | 44.05 | *37.00* | 44.84 | *37.73* | 45.68 | *38.48* | 46.56 |
| 80.0 | 43.85 | *36.82* | 44.24 | *37.17* | 45.04 | *37.90* | 45.88 | *38.67* | 46.77 |

*Alcohol table.*—Continued.

| 21° C. | 22° C. | | 23° C. | | 24° C. | | 25° C. | | SCALE READING |
|---|---|---|---|---|---|---|---|---|---|
| Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | Per cent by volume | Per cent by weight | |
| 35.92 | 43.63 | 36.62 | 44.41 | 37.33 | 45.24 | 38.08 | 46.12 | 38.88 | 76.0 |
| 36.08 | 43.81 | 36.79 | 44.60 | 37.50 | 45.44 | 38.27 | 46.34 | 39.08 | 76.2 |
| 36.25 | 44.00 | 36.96 | 44.80 | 37.68 | 45.65 | 38.46 | 46.56 | 39.29 | 76.4 |
| 36.42 | 44.19 | 37.13 | 44.99 | 37.86 | 45.86 | 38.65 | 46.78 | 39.49 | 76.6 |
| 36.59 | 44.38 | 37.30 | 45.19 | 38.04 | 46.07 | 38.84 | 47.00 | 39.69 | 76.8 |
| 36.76 | 44.57 | 37.47 | 45.40 | 38.23 | 46.29 | 39.03 | 47.23 | 39.90 | 77.0 |
| 36.93 | 44.76 | 37.65 | 45.60 | 38.42 | 46.51 | 39.23 | 47.45 | 40.11 | 77.2 |
| 37.10 | 44.95 | 37.82 | 45.81 | 38.60 | 46.73 | 39.44 | 47.68 | 40.32 | 77.4 |
| 37.28 | 45.15 | 37.99 | 46.01 | 38.79 | 46.95 | 39.64 | 47.91 | 40.54 | 77.6 |
| 37.45 | 45.35 | 38.18 | 46.23 | 38.98 | 47.17 | 39.85 | 48.14 | 40.75 | 77.8 |
| 37.62 | 45.56 | 38.37 | 46.45 | 39.18 | 47.40 | 40.05 | 48.37 | 40.97 | 78.0 |
| 37.79 | 45.76 | 38.56 | 46.67 | 39.39 | 47.63 | 40.27 | 48.60 | 41.18 | 78.2 |
| 37.97 | 45.96 | 38.75 | 46.89 | 39.59 | 47.85 | 40.48 | 48.84 | 41.40 | 78.4 |
| 38.15 | 46.17 | 38.93 | 47.11 | 39.80 | 48.08 | 40.69 | 49.07 | 41.62 | 78.6 |
| 38.33 | 46.39 | 39.13 | 47.34 | 40.00 | 48.31 | 40.90 | 49.31 | 41.84 | 78.8 |
| 38.52 | 46.61 | 39.33 | 47.56 | 40.21 | 48.53 | 41.12 | 49.54 | 42.05 | 79.0 |
| 38.70 | 46.83 | 39.54 | 47.79 | 40.42 | 48.76 | 41.33 | 49.77 | 42.27 | 79.2 |
| 38.89 | 47.04 | 39.74 | 48.01 | 40.63 | 48.99 | 41.54 | 50.01 | 42.49 | 79.4 |
| 39.08 | 47.26 | 39.94 | 48.23 | 40.84 | 49.22 | 41.76 | 50.24 | 42.71 | 79.6 |
| 39.28 | 47.48 | 40.14 | 48.46 | 41.05 | 49.45 | 41.97 | 50.48 | 42.93 | 79.8 |
| 39.48 | 47.70 | 40.35 | 48.68 | 41.26 | 49.68 | 42.18 | 50.71 | 43.15 | 80.0 |

23 *For determining added water in milk by means of the freezing-point depression (based on Winter's table*[1]*).*

(For practical purposes the added water results may be expressed to the nearest decimal.)

| FREEZING POINT OF SAMPLE, BELOW ZERO C. | ADDED WATER, PER CENT BY VOLUME | FREEZING POINT OF SAMPLE, BELOW ZERO C. | ADDED WATER, PER CENT BY VOLUME | FREEZING POINT OF SAMPLE, BELOW ZERO C. | ADDED WATER, PER CENT BY VOLUME |
|---|---|---|---|---|---|
| 0.550 | 0.00 | 0.505 | 8.18 | 0.460 | 16.36 |
| 0.549 | 0.18 | 0.504 | 8.36 | 0.459 | 16.54 |
| 0.548 | 0.36 | 0.503 | 8.54 | 0.458 | 16.73 |
| 0.547 | 0.54 | 0.502 | 8.73 | 0.457 | 16.91 |
| 0.546 | 0.73 | 0.501 | 8.91 | 0.456 | 17.09 |
| 0.545 | 0.91 | 0.500 | 9.09 | 0.455 | 17.27 |
| 0.544 | 1.09 | 0.499 | 9.27 | 0.454 | 17.45 |
| 0.543 | 1.27 | 0.498 | 9.45 | 0.453 | 17.64 |
| 0.542 | 1.45 | 0.497 | 9.64 | 0.452 | 17.82 |
| 0.541 | 1.63 | 0.496 | 9.82 | 0.451 | 18.00 |
| 0.540 | 1.82 | 0.495 | 10.00 | 0.450 | 18.18 |
| 0.539 | 2.00 | 0.494 | 10.18 | 0.449 | 18.36 |
| 0.538 | 2.18 | 0.493 | 10.36 | 0.448 | 18.54 |
| 0.537 | 2.36 | 0.492 | 10.54 | 0.447 | 18.73 |
| 0.536 | 2.54 | 0.491 | 10.72 | 0.446 | 18.91 |
| 0.535 | 2.72 | 0.490 | 10.91 | 0.445 | 19.09 |
| 0.534 | 2.91 | 0.489 | 11.09 | 0.444 | 19.27 |
| 0.533 | 3.09 | 0.488 | 11.27 | 0.443 | 19.45 |
| 0.532 | 3.27 | 0.487 | 11.45 | 0.442 | 19.64 |
| 0.531 | 3.45 | 0.486 | 11.64 | 0.441 | 19.82 |
| 0.530 | 3.64 | 0.485 | 11.82 | 0.440 | 20.00 |
| 0.529 | 3.82 | 0.484 | 12.00 | 0.439 | 20.18 |
| 0.528 | 4.00 | 0.483 | 12.18 | 0.438 | 20.36 |
| 0.527 | 4.18 | 0.482 | 12.36 | 0.437 | 20.54 |
| 0.526 | 4.36 | 0.481 | 12.54 | 0.436 | 20.73 |
| 0.525 | 4.54 | 0.480 | 12.73 | 0.435 | 20.91 |
| 0.524 | 4.73 | 0.479 | 12.91 | 0.434 | 21.09 |
| 0.523 | 4.91 | 0.478 | 13.09 | 0.433 | 21.27 |
| 0.522 | 5.09 | 0.477 | 13.27 | 0.432 | 21.45 |
| 0.521 | 5.27 | 0.476 | 13.45 | 0.431 | 21.64 |
| 0.520 | 5.45 | 0.475 | 13.64 | 0.430 | 21.82 |
| 0.519 | 5.63 | 0.474 | 13.82 | 0.429 | 22.00 |
| 0.518 | 5.82 | 0.473 | 14.00 | 0.428 | 22.18 |
| 0.517 | 6.00 | 0.472 | 14.18 | 0.427 | 22.36 |
| 0.516 | 6.18 | 0.471 | 14.37 | 0.426 | 22.54 |
| 0.515 | 6.36 | 0.470 | 14.54 | 0.425 | 22.73 |
| 0.514 | 6.54 | 0.469 | 14.73 | 0.424 | 22.91 |
| 0.513 | 6.73 | 0.468 | 14.91 | 0.423 | 23.09 |
| 0.512 | 6.91 | 0.467 | 15.09 | 0.422 | 23.27 |
| 0.511 | 7.09 | 0.466 | 15.27 | 0.421 | 23.45 |
| 0.510 | 7.27 | 0.465 | 15.45 | 0.420 | 23.64 |
| 0.509 | 7.45 | 0.464 | 15.63 | 0.419 | 23.82 |
| 0.508 | 7.64 | 0.463 | 15.82 | 0.418 | 24.00 |
| 0.507 | 7.82 | 0.462 | 16.00 | 0.417 | 24.18 |
| 0.506 | 8.00 | 0.461 | 16.18 | 0.416 | 24.36 |

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[1] Chem. News, **110**, 283 (1914).

*For determining added water in milk by means of the freezing-point depression (based on Winter's table).*—Concluded. 23

| FREEZING POINT OF SAMPLE, BELOW ZERO C. | ADDED WATER, PER CENT BY VOLUME | FREEZING POINT OF SAMPLE, BELOW ZERO C. | ADDED WATER, PER CENT BY VOLUME | FREEZING POINT OF SAMPLE, BELOW ZERO C. | ADDED WATER, PER CENT BY VOLUME |
|---|---|---|---|---|---|
| 0.415 | 24.54 | 0.390 | 29.09 | 0.365 | 33.64 |
| 0.414 | 24.73 | 0.389 | 29.27 | 0.364 | 33.82 |
| 0.413 | 24.91 | 0.388 | 29.45 | 0.363 | 34.00 |
| 0.412 | 25.09 | 0.387 | 29.64 | 0.362 | 34.18 |
| 0.411 | 25.27 | 0.386 | 29.82 | 0.361 | 34.36 |
| 0.410 | 25.45 | 0.385 | 30.00 | 0.360 | 34.54 |
| 0.409 | 25.64 | 0.384 | 30.18 | 0.359 | 34.73 |
| 0.408 | 25.82 | 0.383 | 30.36 | 0.358 | 34.91 |
| 0.407 | 26.00 | 0.382 | 30.54 | 0.357 | 35.09 |
| 0.406 | 26.18 | 0.381 | 30.73 | 0.356 | 35.27 |
| 0.405 | 26.36 | 0.380 | 30.91 | 0.355 | 35.45 |
| 0.404 | 26.54 | 0.379 | 31.09 | 0.354 | 35.64 |
| 0.403 | 26.73 | 0.378 | 31.27 | 0.353 | 35.82 |
| 0.402 | 26.91 | 0.377 | 31.45 | 0.352 | 36.00 |
| 0.401 | 27.09 | 0.376 | 31.64 | 0.351 | 36.18 |
| 0.400 | 27.27 | 0.375 | 31.82 | 0.350 | 36.36 |
| 0.399 | 27.45 | 0.374 | 32.00 | | |
| 0.398 | 27.64 | 0.373 | 32.18 | | |
| 0.397 | 27.82 | 0.372 | 32.36 | | |
| 0.396 | 28.00 | 0.371 | 32.54 | | |
| 0.395 | 28.18 | 0.370 | 32.73 | | |
| 0.394 | 28.36 | 0.369 | 32.91 | | |
| 0.393 | 28.54 | 0.368 | 33.09 | | |
| 0.392 | 28.73 | 0.367 | 33.27 | | |
| 0.391 | 28.91 | 0.366 | 33.45 | | |

## 24

## *Density of carbon dioxide (Parr).*[1]

(Weight in milligrams of 1 cc of carbon dioxide at 700–770 mm pressure and 10–30°C. Corrected for aqueous vapor and barometer readings on glass scale. Calculated from 1.976 equals weight of liter of $CO_2$ at 0°C., 760 mm pressure and 41° latitude.)

| mm. | 10° | 11° | 12° | 13° | 14° | 15° | 16° | 17° | 18° | 19° |
|---|---|---|---|---|---|---|---|---|---|---|
| 700 | 1.7288 | 1.7201 | 1.7113 | 1.7020 | 1.6927 | 1.6863 | 1.6799 | 1.6716 | 1.6632 | 1.6547 |
| 702 | 1.7338 | 1.7252 | 1.7164 | 1.7072 | 1.6980 | 1.6914 | 1.6848 | 1.6765 | 1.6680 | 1.6595 |
| 704 | 1.7388 | 1.7302 | 1.7215 | 1.7124 | 1.7033 | 1.6965 | 1.6897 | 1.6813 | 1.6729 | 1.6644 |
| 706 | 1.7438 | 1.7353 | 1.7266 | 1.7176 | 1.7086 | 1.7016 | 1.6946 | 1.6862 | 1.6778 | 1.6692 |
| 708 | 1.7488 | 1.7403 | 1.7317 | 1.7228 | 1.7139 | 1.7067 | 1.6995 | 1.6911 | 1.6826 | 1.6741 |
| 710 | 1.7538 | 1.7453 | 1.7368 | 1.7280 | 1.7192 | 1.7118 | 1.7044 | 1.6960 | 1.6874 | 1.6789 |
| 712 | 1.7588 | 1.7504 | 1.7419 | 1.7332 | 1.7245 | 1.7169 | 1.7092 | 1.7008 | 1.6922 | 1.6837 |
| 714 | 1.7638 | 1.7555 | 1.7470 | 1.7384 | 1.7298 | 1.7220 | 1.7141 | 1.7057 | 1.6970 | 1.6886 |
| 716 | 1.7688 | 1.7605 | 1.7521 | 1.7436 | 1.7351 | 1.7271 | 1.7190 | 1.7106 | 1.7019 | 1.6934 |
| 718 | 1.7738 | 1.7656 | 1.7572 | 1.7488 | 1.7404 | 1.7322 | 1.7239 | 1.7154 | 1.7068 | 1.6983 |
| 720 | 1.7788 | 1.7706 | 1.7623 | 1.7540 | 1.7457 | 1.7373 | 1.7288 | 1.7203 | 1.7117 | 1.7031 |
| 722 | 1.7838 | 1.7756 | 1.7673 | 1.7590 | 1.7506 | 1.7422 | 1.7337 | 1.7252 | 1.7166 | 1.7079 |
| 724 | 1.7888 | 1.7806 | 1.7723 | 1.7639 | 1.7555 | 1.7471 | 1.7386 | 1.7301 | 1.7215 | 1.7128 |
| 726 | 1.7938 | 1.7856 | 1.7773 | 1.7689 | 1.7605 | 1.7520 | 1.7435 | 1.7349 | 1.7263 | 1.7176 |
| 728 | 1.7988 | 1.7905 | 1.7822 | 1.7738 | 1.7654 | 1.7569 | 1.7484 | 1.7398 | 1.7312 | 1.7225 |
| 730 | 1.8038 | 1.7955 | 1.7872 | 1.7788 | 1.7703 | 1.7618 | 1.7533 | 1.7447 | 1.7360 | 1.7273 |
| 732 | 1.8089 | 1.8005 | 1.7921 | 1.7837 | 1.7752 | 1.7667 | 1.7582 | 1.7496 | 1.7409 | 1.7321 |
| 734 | 1.8139 | 1.8055 | 1.7971 | 1.7887 | 1.7802 | 1.7717 | 1.7631 | 1.7545 | 1.7458 | 1.7370 |
| 736 | 1.8189 | 1.8105 | 1.8021 | 1.7936 | 1.7851 | 1.7766 | 1.7680 | 1.7593 | 1.7506 | 1.7418 |
| 738 | 1.8239 | 1.8155 | 1.8071 | 1.7986 | 1.7901 | 1.7815 | 1.7729 | 1.7642 | 1.7555 | 1.7467 |
| 740 | 1.8288 | 1.8204 | 1.8120 | 1.8035 | 1.7950 | 1.7864 | 1.7778 | 1.7691 | 1.7603 | 1.7515 |
| 742 | 1.8338 | 1.8254 | 1.8170 | 1.8085 | 1.7999 | 1.7913 | 1.7827 | 1.7740 | 1.7652 | 1.7564 |
| 744 | 1.8388 | 1.8304 | 1.8219 | 1.8134 | 1.8048 | 1.7962 | 1.7875 | 1.7788 | 1.7700 | 1.7612 |
| 746 | 1.8439 | 1.8354 | 1.8269 | 1.8184 | 1.8098 | 1.8011 | 1.7924 | 1.7837 | 1.7749 | 1.7661 |
| 748 | 1.8489 | 1.8404 | 1.8319 | 1.8233 | 1.8147 | 1.8060 | 1.7973 | 1.7886 | 1.7798 | 1.7709 |
| 750 | 1.8539 | 1.8454 | 1.8368 | 1.8282 | 1.8196 | 1.8109 | 1.8022 | .17934 | 1.7846 | 1.7757 |
| 752 | 1.8589 | 1.8504 | 1.8418 | 1.8332 | 1.8246 | 1.8159 | 1.8072 | 1.7984 | 1.7895 | 1.7806 |
| 754 | 1.8639 | 1.8554 | 1.8468 | 1.8382 | 1.8295 | 1.8208 | 1.8120 | 1.8032 | 1.7944 | 1.7854 |
| 756 | 1.8689 | 1.8603 | 1.8517 | 1.8431 | 1.8344 | 1.8257 | 1.8169 | 1.8081 | 1.7992 | 1.7902 |
| 758 | 1.8739 | 1.8653 | 1.8567 | 1.8481 | 1.8394 | 1.8306 | 1.8218 | 1.8130 | 1.8041 | 1.7951 |
| 760 | 1.8789 | 1.8703 | 1.8617 | 1.8530 | 1.8443 | 1.8355 | 1.8267 | 1.8178 | 1.8089 | 1.7999 |
| 762 | 1.8839 | 1.8753 | 1.8667 | 1.8580 | 1.8492 | 1.8404 | 1.8316 | 1.8227 | 1.8138 | 1.8048 |
| 764 | 1.8890 | 1.8803 | 1.8716 | 1.8629 | 1.8541 | 1.8453 | 1.8365 | 1.8276 | 1.8137 | 1.8096 |
| 766 | 1.8940 | 1.8853 | 1.8766 | 1.8679 | 1.8591 | 1.8503 | 1.8414 | 1.8325 | 1.8235 | 1.8144 |
| 768 | 1.8990 | 1.8903 | 1.8816 | 1.8728 | 1.8640 | 1.8552 | 1.8463 | 1.8374 | 1.8284 | 1.8193 |
| 770 | 1.9040 | 1.8953 | 1.8865 | 1.8777 | 1.8689 | 1.8601 | 1.8512 | 1.8422 | 1.8332 | 1.8241 |

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[1] J. Am Chem. Soc., **31**, 237 (1900). The values for 700–718 mm. have been calculated by formula given by Parr.

### *Density of carbon dioxide (Parr).*[1]—Concluded 24

| 20° | 21° | 22° | 23° | 24° | 25° | 26° | 27° | 28° | 29° | 30° | mm. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.6462 | 1.6370 | 1.6278 | 1.6195 | 1.6112 | 1.6021 | 1.5930 | 1.5837 | 1.5744 | 1.5649 | 1.5554 | 700 |
| 1.6510 | 1.6419 | 1.6327 | 1.6243 | 1.6160 | 1.6068 | 1.5977 | 1.5884 | 1.5791 | 1.5696 | 1.5600 | 702 |
| 1.6558 | 1.6467 | 1.6376 | 1.6292 | 1.6207 | 1.6116 | 1.6025 | 1.5931 | 1.5838 | 1.5742 | 1.5647 | 704 |
| 1.6607 | 1.6516 | 1.6425 | 1.6340 | 1.6254 | 1.6163 | 1.6072 | 1.5979 | 1.5885 | 1.5789 | 1.5693 | 706 |
| 1.6655 | 1.6564 | 1.6474 | 1.6388 | 1.6302 | 1.6211 | 1.6119 | 1.6026 | 1.5932 | 1.5836 | 1.5740 | 708 |
| 1.6703 | 1.6613 | 1.6522 | 1.6436 | 1.6350 | 1.6258 | 1.6166 | 1.6073 | 1.5978 | 1.5882 | 1.5786 | 710 |
| 1.6751 | 1.6662 | 1.6571 | 1.6485 | 1.6397 | 1.6305 | 1.6214 | 1.6120 | 1.6025 | 1.5929 | 1.5832 | 712 |
| 1.6799 | 1.6710 | 1.6620 | 1.6533 | 1.6444 | 1.6353 | 1.6261 | 1.6167 | 1.6072 | 1.5976 | 1.5879 | 714 |
| 1.6848 | 1.6759 | 1.6670 | 1.6581 | 1.6492 | 1.6400 | 1.6308 | 1.6215 | 1.6119 | 1.6023 | 1.5925 | 716 |
| 1.6896 | 1.6807 | 1.6718 | 1.6629 | 1.6540 | 1.6448 | 1.6356 | 1.6262 | 1.6166 | 1.6069 | 1.5972 | 818 |
| 1.6944 | 1.6856 | 1.6767 | 1.6678 | 1.6587 | 1.6495 | 1.6403 | 1.6309 | 1.6213 | 1.6116 | 1.6018 | 720 |
| 1.6992 | 1.6904 | 1.6815 | 1.6726 | 1.6635 | 1.6543 | 1.6450 | 1.6356 | 1.6260 | 1.6163 | 1.6065 | 722 |
| 1.7041 | 1.6953 | 1.6863 | 1.6773 | 1.6682 | 1.6590 | 1.6497 | 1.6403 | 1.6307 | 1.6210 | 1.6111 | 724 |
| 1.7089 | 1.7001 | 1.6911 | 1.6821 | 1.6730 | 1.6638 | 1.6544 | 1.6450 | 1.6354 | 1.6256 | 1.6157 | 726 |
| 1.7137 | 1.7049 | 1.6959 | 1.6869 | 1.6778 | 1.6685 | 1.6591 | 1.6497 | 1.6401 | 1.6303 | 1.6204 | 728 |
| 1.7185 | 1.7097 | 1.7007 | 1.6917 | 1.6825 | 1.6732 | 1.6638 | 1.5544 | 1.6448 | 1.6350 | 1.6251 | 730 |
| 1.7233 | 1.7145 | 1.7055 | 1.6964 | 1.6872 | 1.6779 | 1.6685 | 1.6591 | 1.6494 | 1.6396 | 1.6297 | 732 |
| 1.7282 | 1.7193 | 1.7103 | 1.7012 | 1.6920 | 1.6827 | 1.6733 | 1.6638 | 1.6541 | 1.6443 | 1.6343 | 734 |
| 1.7330 | 1.7241 | 1.7151 | 1.7060 | 1.6968 | 1.6875 | 1.6780 | 1.6685 | 1.6588 | 1.6490 | 1.6390 | 736 |
| 1.7378 | 1.7289 | 1.7199 | 1.7107 | 1.7015 | 1.6922 | 1.6827 | 1.6732 | 1.6635 | 1.6537 | 1.6437 | 738 |
| 1.7426 | 1.7337 | 1.7247 | 1.7155 | 1.7063 | 1.6969 | 1.6874 | 1.6778 | 1.6681 | 1.6583 | 1.6483 | 740 |
| 1.7475 | 1.7385 | 1.7295 | 1.7203 | 1.7111 | 1.7017 | 1.6922 | 1.6826 | 1.6729 | 1.6630 | 1.6530 | 742 |
| 1.7523 | 1.7433 | 1.7342 | 1.7250 | 1.7158 | 1.7064 | 1.6969 | 1.6873 | 1.6776 | 1.6677 | 1.6577 | 744 |
| 1.7571 | 1.7481 | 1.7390 | 1.7298 | 1.7206 | 1.7112 | 1.7016 | 1.6920 | 1.6822 | 1.6723 | 1.6623 | 746 |
| 1.7619 | 1.7529 | 1.7438 | 1.7346 | 1.7253 | 1.7159 | 1.7063 | 1.6967 | 1.6869 | 1.6770 | 1.6670 | 748 |
| 1.7667 | 1.7577 | 1.7486 | 1.7394 | 1.7301 | 1.7206 | 1.7110 | 1.7014 | 1.6916 | 1.6817 | 1.6716 | 750 |
| 1.7716 | 1.7625 | 1.7534 | 1.7441 | 1.7348 | 1.7254 | 1.7158 | 1.7061 | 1.6963 | 1.6864 | 1.6763 | 752 |
| 1.7764 | 1.7673 | 1.7582 | 1.7489 | 1.7396 | 1.7301 | 1.7205 | 1.7108 | 1.7010 | 1.6910 | 1.6809 | 754 |
| 1.7812 | 1.7721 | 1.7630 | 1.7537 | 1.7443 | 1.7348 | 1.7252 | 1.7155 | 1.7057 | 1.6957 | 1.6856 | 756 |
| 1.7861 | 1.7770 | 1.7678 | 1.7585 | 1.7491 | 1.7396 | 1.7300 | 1.7202 | 1.7104 | 1.7004 | 1.6903 | 758 |
| 1.7909 | 1.7818 | 1.7725 | 1.7632 | 1.7538 | 1.7443 | 1.7347 | 1.7249 | 1.7150 | 1.7050 | 1.6949 | 760 |
| 1.7957 | 1.7866 | 1.7773 | 1.7680 | 1.7586 | 1.7490 | 1.7394 | 1.7296 | 1.7197 | 1.7097 | 1.6996 | 762 |
| 1.8005 | 1.7914 | 1.7821 | 1.7728 | 1.7633 | 1.7538 | 1.7441 | 1.7343 | 1.7244 | 1.7144 | 1.7042 | 764 |
| 1.8053 | 1.7962 | 1.7869 | 1.7776 | 1.7681 | 1.7585 | 1.7488 | 1.7390 | 1.7291 | 1.7191 | 1.7089 | 766 |
| 1.8102 | 1.8010 | 1.7917 | 1.7823 | 1.7728 | 1.7633 | 1.7535 | 1.7437 | 1.7338 | 1.7237 | 1.7135 | 768 |
| 1.8150 | 1.8058 | 1.7965 | 1.7871 | 1.7776 | 1.7680 | 1.7582 | 1.7484 | 1.7385 | 1.7284 | 1.7182 | 770 |

## 25

## *Correction factors for the gasometric determination of carbon dioxide.*

(Based on sample weighing 1.7000 grams.)

| °C. | 15.0° | 15.5° | 16.0° | 16.5° | 17.0° | 17.5° | 18.0° | 18.5° | |
|---|---|---|---|---|---|---|---|---|---|
| *mm.* | | | | | | | | | *inches.* |
| 700 | 0.99194 | 0.99006 | 0.98818 | 0.98573 | 0.98329 | 0.98082 | 0.97835 | 0.97585 | 27.56 |
| 702 | 0.99494 | 0.99300 | 0.99106 | 0.98862 | 0.98618 | 0.98368 | 0.98118 | 0.97868 | 27.64 |
| 704 | 0.99794 | 0.99544 | 0.99394 | 0.99147 | 0.98900 | 0.98653 | 0.98406 | 0.98156 | 27.72 |
| 706 | 1.00094 | 0.99886 | 0.99682 | 0.99435 | 0.99188 | 0.98941 | 0.98694 | 0.98406 | 27.80 |
| 708 | 1.00394 | 1.00183 | 0.99971 | 0.99723 | 0.99476 | 0.99226 | 0.98976 | 0.98726 | 27.87 |
| 710 | 1.00694 | 1.00477 | 1.00259 | 1.00012 | 0.99765 | 0.99512 | 0.99259 | 0.99009 | 27.95 |
| 712 | 1.00994 | 1.00767 | 1.00541 | 1.00294 | 1.00047 | 0.99795 | 0.99541 | 0.99291 | 28.03 |
| 714 | 1.01294 | 1.01061 | 1.00829 | 1.00582 | 1.00335 | 1.00080 | 0.99824 | 0.99576 | 28.11 |
| 716 | 1.01594 | 1.01356 | 1.01118 | 1.00871 | 1.00624 | 1.00368 | 1.00112 | 0.99861 | 28.19 |
| 718 | 1.01894 | 1.01650 | 1.01406 | 1.01156 | 1.00906 | 1.00653 | 1.00400 | 1.00150 | 28.27 |
| 720 | 1.02194 | 1.01949 | 1.01694 | 1.01444 | 1.01194 | 1.00941 | 1.00688 | 1.00435 | 28.35 |
| 722 | 1.02482 | 1.02232 | 1.01982 | 1.01732 | 1.01482 | 1.01229 | 1.00976 | 1.00720 | 28.43 |
| 724 | 1.02771 | 1.02521 | 1.02271 | 1.02021 | 1.01771 | 1.01518 | 1.01265 | 1.01009 | 28.50 |
| 726 | 1.03059 | 1.02809 | 1.02559 | 1.02306 | 1.02053 | 1.01800 | 1.01574 | 1.01291 | 28.58 |
| 728 | 1.03347 | 1.03097 | 1.02847 | 1.02594 | 1.02341 | 1.02088 | 1.01835 | 1.01580 | 28.66 |
| 730 | 1.03635 | 1.03385 | 1.03135 | 1.02882 | 1.02629 | 1.02374 | 1.02118 | 1.01862 | 28.74 |
| 732 | 1.03924 | 1.03674 | 1.03424 | 1.03171 | 1.02918 | 1.02662 | 1.02406 | 1.02147 | 28.82 |
| 734 | 1.04218 | 1.03915 | 1.03712 | 1.03459 | 1.03206 | 1.02950 | 1.02694 | 1.02435 | 28.90 |
| 736 | 1.04506 | 1.04253 | 1.04000 | 1.03744 | 1.03488 | 1.03232 | 1.02976 | 1.02718 | 28.98 |
| 738 | 1.04794 | 1.04541 | 1.04288 | 1.04037 | 1.03776 | 1.03521 | 1.03265 | 1.03006 | 29.06 |
| 740 | 1.05082 | 1.04829 | 1.04576 | 1.04321 | 1.04065 | 1.03806 | 1.03547 | 1.03288 | 29.13 |
| 742 | 1.05371 | 1.05118 | 1.04865 | 1.04609 | 1.04353 | 1.04094 | 1.03835 | 1.03577 | 29.21 |
| 744 | 1.05659 | 1.05403 | 1.05147 | 1.04991 | 1.04635 | 1.04377 | 1.04118 | 1.03859 | 29.29 |
| 746 | 1.05947 | 1.05691 | 1.05435 | 1.05180 | 1.04924 | 1.04665 | 1.04406 | 1.04147 | 29.37 |
| 748 | 1.06235 | 1.05929 | 1.05724 | 1.05418 | 1.05212 | 1.04953 | 1.04694 | 1.04433 | 29.45 |
| 750 | 1.06524 | 1.06218 | 1.06012 | 1.05748 | 1.05494 | 1.05235 | 1.04976 | 1.04715 | 29.53 |
| 752 | 1.06818 | 1.06512 | 1.06306 | 1.06047 | 1.05788 | 1.05527 | 1.05265 | 1.05003 | 29.61 |
| 754 | 1.07106 | 1.06847 | 1.06588 | 1.06330 | 1.06071 | 1.05812 | 1.05553 | 1.05289 | 29.69 |
| 756 | 1.07394 | 1.07135 | 1.06876 | 1.06618 | 1.06359 | 1.06197 | 1.05835 | 1.05571 | 29.76 |
| 758 | 1.07682 | 1.07423 | 1.07165 | 1.06906 | 1.06647 | 1.06386 | 1.06124 | 1.05859 | 29.84 |
| 760 | 1.07971 | 1.07712 | 1.07453 | 1.07191 | 1.06929 | 1.06668 | 1.06406 | 1.06141 | 29.92 |
| 762 | 1.08259 | 1.08050 | 1.07741 | 1.07480 | 1.07218 | 1.06956 | 1.06694 | 1.06430 | 30.00 |
| 764 | 1.08547 | 1.08288 | 1.08029 | 1.07768 | 1.07506 | 1.07244 | 1.06982 | 1.06715 | 30.08 |
| 766 | 1.08841 | 1.08580 | 1.08318 | 1.08056 | 1.07794 | 1.07530 | 1.07265 | 1.06997 | 30.16 |
| 768 | 1.09129 | 1.08868 | 1.08606 | 1.08344 | 1.08082 | 1.07818 | 1.07553 | 1.07285 | 30.24 |
| 770 | 1.09418 | 1.09156 | 1.08894 | 1.08630 | 1.08365 | 1.08100 | 1.07835 | 1.07567 | 30.31 |
| °F. | 59.0° | 59.9° | 60.8° | 61.7° | 62.6° | 63.5° | 64.4° | 65.3° | |

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[1] Calculated from 1.976 = weight of 1 liter $CO_2$ at 0°C., 760 mm pressure and 41° latitude. Formula given by S. W. Parr, *J. Am. Chem. Soc.*, **31:** 237 (1909).

*Correction factors for the gasometric determination of carbon dioxide.*—Continued. **25**

| °C. | 19.0° | 19.5° | 20.0° | 20.5° | 21.0 ° | 21.5° | 22.0° | 22.5° | |
|---|---|---|---|---|---|---|---|---|---|
| *mm.* | | | | | | | | | *inches* |
| 700 | 0.97335 | 0.97085 | 0.96835 | 0.96564 | 0.96294 | 0.96023 | 0.95753 | 0.95509 | 27.56 |
| 702 | 0.97618 | 0.97368 | 0.97118 | 0.96850 | 0.96582 | 0.96311 | 0.96041 | 0.95794 | 27.64 |
| 704 | 0.97906 | 0.97653 | 0.97400 | 0.97132 | 0.96865 | 0.96597 | 0.96329 | 0.96082 | 27.72 |
| 706 | 0.98188 | 0.97938 | 0.97688 | 0.97420 | 0.97153 | 0.96888 | 0.96624 | 0.96371 | 27.80 |
| 708 | 0.98476 | 0.98224 | 0.97971 | 0.97703 | 0.97435 | 0.97173 | 0.96912 | 0.96656 | 27.87 |
| 710 | 0.98759 | 0.98506 | 0.98253 | 0.97988 | 0.97724 | 0.97459 | 0.97195 | 0.96938 | 27.95 |
| 712 | 0.99041 | 0.98788 | 0.98535 | 0.98273 | 0.98012 | 0.97747 | 0.97483 | 0.97227 | 28.03 |
| 714 | 0.99329 | 0.99073 | 0.98818 | 0.98556 | 0.98294 | 0.98032 | 0.97771 | 0.97512 | 28.11 |
| 716 | 0.99612 | 0.99358 | 0.99106 | 0.98844 | 0.98582 | 0.98323 | 0.98065 | 0.97800 | 28.19 |
| 718 | 0.99900 | 0.99644 | 0.99388 | 0.99126 | 0.98865 | 0.98606 | 0.98348 | 0.98083 | 28.27 |
| 720 | 1.00182 | 0.99925 | 0.99671 | 0.99412 | 0.99153 | 0.98894 | 0.98636 | 0.98371 | 28.35 |
| 722 | 1.00465 | 1.00209 | 0.99953 | 0.99694 | 0.99435 | 0.99176 | 0.98918 | 0.98653 | 28.43 |
| 724 | 1.00753 | 1.00497 | 1.00241 | 0.99982 | 0.99724 | 0.99462 | 0.99200 | 0.98932 | 28.50 |
| 726 | 1.01035 | 1.00779 | 1.00524 | 1.00265 | 1.00006 | 0.99746 | 0.99483 | 0.99215 | 28.58 |
| 728 | 1.01324 | 1.01065 | 1.00806 | 1.00547 | 1.00288 | 1.00027 | 0.99765 | 0.99497 | 28.66 |
| 730 | 1.01606 | 1.01347 | 1.01088 | 1.00829 | 1.00571 | 1.00306 | 1.00041 | 0.99781 | 28.74 |
| 732 | 1.01888 | 1.01629 | 1.01371 | 1.01112 | 1.00853 | 1.00588 | 1.00324 | 1.00056 | 28.82 |
| 734 | 1.02176 | 1.01919 | 1.01659 | 1.01497 | 1.01135 | 1.00870 | 1.00606 | 1.00338 | 28.90 |
| 736 | 1.02459 | 1.02200 | 1.01941 | 1.01679 | 1.01418 | 1.01153 | 1.00888 | 1.00620 | 28.98 |
| 738 | 1.02747 | 1.02486 | 1.02224 | 1.01962 | 1.01700 | 1.01435 | 1.01171 | 1.00900 | 29.06 |
| 740 | 1.03029 | 1.02768 | 1.02506 | 1.02244 | 1.01982 | 1.01717 | 1.01453 | 1.01182 | 29.13 |
| 742 | 1.03318 | 1.03056 | 1.02794 | 1.02529 | 1.02265 | 1.02000 | 1.01735 | 1.01464 | 29.21 |
| 744 | 1.03600 | 1.03338 | 1.03076 | 1.02811 | 1.02547 | 1.02279 | 1.02212 | 1.01752 | 29.29 |
| 746 | 1.03888 | 1.03624 | 1.03359 | 1.03094 | 1.02829 | 1.02561 | 1.02294 | 1.02024 | 29.37 |
| 748 | 1.04171 | 1.03906 | 1.03641 | 1.03376 | 1.03112 | 1.02844 | 1.02576 | 1.02306 | 29.45 |
| 750 | 1.04453 | 1.04189 | 1.03924 | 1.03659 | 1.03394 | 1.03126 | 1.02859 | 1.02589 | 29.53 |
| 752 | 1.04741 | 1.04477 | 1.04212 | 1.03944 | 1.03676 | 1.03408 | 1.03141 | 1.02868 | 29.61 |
| 754 | 1.05024 | 1.04759 | 1.04494 | 1.04226 | 1.03959 | 1.03691 | 1.03424 | 1.03150 | 29.69 |
| 756 | 1.05306 | 1.05041 | 1.04776 | 1.04508 | 1.04241 | 1.03973 | 1.03706 | 1.03433 | 29.76 |
| 758 | 1.05594 | 1.05330 | 1.05065 | 1.04797 | 1.04529 | 1.04259 | 1.03988 | 1.03715 | 29.84 |
| 760 | 1.05876 | 1.05612 | 1.05347 | 1.05079 | 1.04812 | 1.04539 | 1.04265 | 1.03992 | 29.92 |
| 762 | 1.06165 | 1.05897 | 1.05629 | 1.05361 | 1.05094 | 1.04821 | 1.04547 | 1.04274 | 30.00 |
| 764 | 1.06447 | 1.06179 | 1.05912 | 1.05644 | 1.05376 | 1.05103 | 1.04829 | 1.04556 | 30.08 |
| 766 | 1.06729 | 1.06462 | 1.06194 | 1.05926 | 1.05659 | 1.05386 | 1.05112 | 1.04839 | 30.16 |
| 768 | 1.07018 | 1.06750 | 1.06482 | 1.06212 | 1.05941 | 1.05668 | 1.05394 | 1.05118 | 30.24 |
| 770 | 1.07300 | 1.07032 | 1.06765 | 1.06424 | 1.06224 | 1.05950 | 1.05676 | 1.05400 | 30.31 |
| °F. | 66.2° | 67.1° | 68.0° | 68.9° | 69.8° | 70.7° | 71.6° | 72.5° | |

25 *Correction factors for the gasometric determination of carbon dioxide.*—Continued.

| °C. | 23.0° | 23.5° | 24.0° | 24.5° | 25.0° | 25.5° | 26.0° | 26.5° | |
|---|---|---|---|---|---|---|---|---|---|
| *mm.* | | | | | | | | | *inches* |
| 700 | 0.95265 | 0.95020 | 0.94776 | 0.94508 | 0.94241 | 0.93973 | 0.93706 | 0.93432 | 27.56 |
| 702 | 0.95547 | 0.95303 | 0.95059 | 0.94788 | 0.94518 | 0.94250 | 0.93982 | 0.93708 | 27.64 |
| 704 | 0.95835 | 0.95585 | 0.95335 | 0.95067 | 0.94800 | 0.94532 | 0.94265 | 0.93988 | 27.72 |
| 706 | 0.96118 | 0.95865 | 0.95612 | 0.95344 | 0.95076 | 0.94808 | 0.94541 | 0.94267 | 27.80 |
| 708 | 0.96400 | 0.96147 | 0.95894 | 0.95626 | 0.95359 | 0.95088 | 0.94818 | 0.94544 | 27.87 |
| 710 | 0.96682 | 0.96429 | 0.96176 | 0.95905 | 0.95635 | 0.95364 | 0.95094 | 0.94820 | 27.95 |
| 712 | 0.96971 | 0.96712 | 0.96453 | 0.96182 | 0.95912 | 0.95644 | 0.95376 | 0.95100 | 28.03 |
| 714 | 0.97253 | 0.96991 | 0.96729 | 0.96461 | 0.96194 | 0.95923 | 0.95653 | 0.95376 | 28.11 |
| 716 | 0.97535 | 0.97273 | 0.97012 | 0.96741 | 0.96471 | 0.96200 | 0.95929 | 0.95655 | 28.19 |
| 718 | 0.97818 | 0.97556 | 0.97294 | 0.97023 | 0.96753 | 0.96482 | 0.96212 | 0.95935 | 28.27 |
| 720 | 0.98106 | 0.97838 | 0.97571 | 0.97300 | 0.97029 | 0.96758 | 0.96488 | 0.96213 | 28.35 |
| 722 | 0.98388 | 0.98120 | 0.97853 | 0.97582 | 0.97312 | 0.97038 | 0.96765 | 0.96488 | 28.43 |
| 724 | 0.98665 | 0.98397 | 0.98129 | 0.97858 | 0.97588 | 0.97314 | 0.97041 | 0.96764 | 28.50 |
| 726 | 0.98947 | 0.98679 | 0.98412 | 0.98141 | 0.97871 | 0.97594 | 0.97318 | 0.97041 | 28.58 |
| 728 | 0.99229 | 0.98961 | 0.98694 | 0.98420 | 0.08147 | 0.97870 | 0.97594 | 0.97319 | 28.66 |
| 730 | 0.99512 | 0.99241 | 0.98971 | 0.98697 | 0.98424 | 0.98147 | 0.97871 | 0.97594 | 28.74 |
| 732 | 0.99788 | 0.99517 | 0.99247 | 0.98973 | 0.98700 | 0.98423 | 0.98147 | 0.97871 | 28.82 |
| 734 | 1.00071 | 0.99799 | 0.99529 | 0.99255 | 0.98982 | 0.98705 | 0.98429 | 0.98165 | 28.90 |
| 736 | 1.00353 | 1.00083 | 0.99812 | 0.99538 | 0.99265 | 0.98985 | 0.98706 | 0.98426 | 28.98 |
| 738 | 1.00629 | 1.00359 | 1.00088 | 0.99815 | 0.99541 | 0.99261 | 0.98982 | 0.98703 | 29.06 |
| 740 | 1.00912 | 1.00643 | 1.00371 | 1.00095 | 0.99818 | 0.99538 | 0.99259 | 0.98976 | 29.13 |
| 742 | 1.01194 | 1.00923 | 1.00653 | 1.00377 | 1.00100 | 0.99820 | 0.99541 | 0.99258 | 29.21 |
| 744 | 1.01471 | 1.01200 | 1.00929 | 1.00643 | 1.00376 | 1.00097 | 0.99818 | 0.99535 | 29.29 |
| 746 | 1.01753 | 1.01482 | 1.01212 | 1.00936 | 1.00659 | 1.00376 | 1.00094 | 0.99809 | 29.37 |
| 748 | 1.02035 | 1.01762 | 1.01488 | 1.01212 | 1.00935 | 1.00653 | 1.00371 | 1.00088 | 29.45 |
| 750 | 1.02318 | 1.02045 | 1.01771 | 1.01492 | 1.01212 | 1.00936 | 1.00659 | 1.00370 | 29.53 |
| 752 | 1.02594 | 1.02321 | 1.02047 | 1.01771 | 1.01494 | 1.01211 | 1.00929 | 1.00644 | 29.61 |
| 754 | 1.02876 | 1.02603 | 1.02329 | 1.02050 | 1.01771 | 1.01483 | 1.01206 | 1.00921 | 29.69 |
| 756 | 1.03159 | 1.02883 | 1.02606 | 1.02326 | 1.02047 | 1.01764 | 1.01482 | 1.01197 | 29.76 |
| 758 | 1.03441 | 1.03165 | 1.02888 | 1.02608 | 1.02329 | 1.02047 | 1.01765 | 1.01477 | 29.84 |
| 760 | 1.03718 | 1.03442 | 1.03165 | 1.02886 | 1.02606 | 1.02323 | 1.02041 | 1.01753 | 29.92 |
| 762 | 1.04000 | 1.03724 | 1.03447 | 1.03164 | 1.02882 | 1.02600 | 1.02318 | 1.02030 | 30.00 |
| 764 | 1.04282 | 1.04003 | 1.03723 | 1.03444 | 1.03165 | 1.02880 | 1.02594 | 1.02306 | 30.08 |
| 766 | 1.04565 | 1.04285 | 1.04005 | 1.03723 | 1.03441 | 1.03156 | 1.02871 | 1.02583 | 30.16 |
| 768 | 1.04841 | 1.04562 | 1.04282 | 1.04003 | 1.03724 | 1.03435 | 1.03147 | 1.02859 | 30.24 |
| 770 | 1.05123 | 1.04844 | 1.04564 | 1.04282 | 1.04000 | 1.03712 | 1.03424 | 1.03136 | 30.31 |
| °F. | 73.4° | 74.3° | 75.2° | 76.1° | 77.0° | 77.9° | 78.8° | 79.7° | |

*Correction factors for the gasometric determination of carbon dioxide.*—Continued. **25**

| °C. | 27.0° | 27.5° | 28.0° | 28.5° | 29.0° | 29.5° | 30.0° | 30.5° | |
|---|---|---|---|---|---|---|---|---|---|
| *mm* | | | | | | | | | *inches* |
| 700 | 0.93159 | 0.92885 | 0.92612 | 0.92332 | 0.92053 | 0.91773 | 0.91494 | 0.91203 | 27.56 |
| 702 | 0.93435 | 0.93161 | 0.92888 | 0.92608 | 0.92329 | 0.92047 | 0.91765 | 0.91476 | 27.64 |
| 704 | 0.93712 | 0.93438 | 0.93165 | 0.92882 | 0.92600 | 0.92320 | 0.92041 | 0.91750 | 27.72 |
| 706 | 0.93994 | 0.93717 | 0.93441 | 0.93158 | 0.92876 | 0.92594 | 0.92312 | 0.92024 | 27.80 |
| 708 | 0.94271 | 0.93994 | 0.93718 | 0.93435 | 0.93153 | 0.92870 | 0.92588 | 0.92297 | 27.87 |
| 710 | 0.94547 | 0.94267 | 0.93988 | 0.93706 | 0.93424 | 0.93141 | 0.92859 | 0.92567 | 27.95 |
| 712 | 0.94824 | 0.94544 | 0.94265 | 0.93982 | 0.93700 | 0.93414 | 0.93129 | 0.92841 | 28.03 |
| 714 | 0.95100 | 0.94820 | 0.94541 | 0.94258 | 0.93976 | 0.93691 | 0.93406 | 0.93115 | 28.11 |
| 716 | 0.95382 | 0.95100 | 0.94818 | 0.94535 | 0.94253 | 0.93964 | 0.93676 | 0.93388 | 28.19 |
| 718 | 0.95659 | 0.95376 | 0.95094 | 0.94809 | 0.94524 | 0.94238 | 0.93953 | 0.93662 | 28.27 |
| 720 | 0.95939 | 0.95655 | 0.95371 | 0.95085 | 0.94800 | 0.94512 | 0.94224 | 0.93932 | 28.35 |
| 722 | 0.96212 | 0.95929 | 0.95647 | 0.95361 | 0.95076 | 0.94788 | 0.94500 | 0.94209 | 28.43 |
| 724 | 0.96488 | 0.96206 | 0.95924 | 0.95638 | 0.95353 | 0.95062 | 0.94771 | 0.94479 | 28.50 |
| 726 | 0.96765 | 0.96482 | 0.96200 | 0.95912 | 0.95624 | 0.95332 | 0.95041 | 0.94750 | 28.58 |
| 728 | 0.97041 | 0.96758 | 0.96476 | 0.96188 | 0.95900 | 0.95609 | 0.95318 | 0.95026 | 28.66 |
| 730 | 0.97318 | 0.97036 | 0.96753 | 0.96464 | 0.96176 | 0.95885 | 0.95594 | 0.95300 | 28.74 |
| 732 | 0.97594 | 0.97309 | 0.97024 | 0.96735 | 0.96447 | 0.96156 | 0.95865 | 0.95578 | 28.82 |
| 734 | 0.97871 | 0.97585 | 0.97300 | 0.97012 | 0.96724 | 0.96429 | 0.96135 | 0.95844 | 28.90 |
| 736 | 0.98147 | 0.97861 | 0.97576 | 0.97288 | 0.97000 | 0.96706 | 0.96412 | 0.96118 | 28.98 |
| 738 | 0.98424 | 0.98138 | 0.97853 | 0.97564 | 0.97276 | 0.96982 | 0.96688 | 0.96394 | 29.06 |
| 740 | 0.98694 | 0.98409 | 0.98124 | 0.97835 | 0.97547 | 0.97253 | 0.96959 | 0.96665 | 29.13 |
| 742 | 0.98976 | 0.98691 | 0.98406 | 0.98115 | 0.97824 | 0.97529 | 0.97235 | 0.96941 | 29.21 |
| 744 | 0.99253 | 0.98967 | 0.98682 | 0.98391 | 0.98100 | 0.97806 | 0.97512 | 0.97215 | 29.29 |
| 746 | 0.99529 | 0.99241 | 0.98953 | 0.98662 | 0.98371 | 0.98076 | 0.97782 | 0.97485 | 29.37 |
| 748 | 0.99806 | 0.99517 | 0.99229 | 0.98938 | 0.98647 | 0.98353 | 0.98059 | 0.97762 | 29.45 |
| 750 | 1.00082 | 0.99796 | 0.99506 | 0.99215 | 0.98924 | 0.98626 | 0.98329 | 0.98032 | 29.53 |
| 752 | 1.00359 | 1.00071 | 0.99782 | 0.99491 | 0.99200 | 0.98903 | 0.98606 | 0.98306 | 29.61 |
| 754 | 1.00635 | 1.00342 | 1.00059 | 0.99738 | 0.99471 | 0.99173 | 0.98876 | 0.98579 | 29.69 |
| 756 | 1.00912 | 1.00624 | 1.00335 | 1.00041 | 0.99747 | 0.99450 | 0.99153 | 0.98853 | 29.76 |
| 758 | 1.01188 | 1.00900 | 1.00612 | 1.00318 | 1.00024 | 0.99724 | 0.99429 | 0.99129 | 29.84 |
| 760 | 1.01465 | 1.01174 | 1.00882 | 1.00588 | 1.00294 | 0.99995 | 0.99700 | 0.99400 | 29.92 |
| 762 | 1.01741 | 1.01450 | 1.01159 | 1.00865 | 1.00571 | 1.00274 | 0.99976 | 0.99673 | 30.00 |
| 764 | 1.02018 | 1.01727 | 1.01435 | 1.01141 | 1.00847 | 1.00547 | 1.00247 | 0.99948 | 30.08 |
| 766 | 1.02294 | 1.02003 | 1.01712 | 1.01418 | 1.01124 | 1.00824 | 1.00524 | 1.00221 | 30.16 |
| 768 | 1.02571 | 1.02280 | 1.01988 | 1.01611 | 1.01394 | 1.01094 | 1.00794 | 1.00491 | 30.24 |
| 770 | 1.02847 | 1.02556 | 1.02265 | 1.01968 | 1.01671 | 1.01371 | 1.01071 | 1.00768 | 30.31 |
| °F. | 80.6° | 81.5° | 82.4° | 83.3° | 84.2° | 85.1° | 86.0° | 86.9° | |

25 *Correction factors for the gasometric determination of carbon dioxide.*—Concluded.

| °C. | 31.0° | 31.5° | 32.0° | 32.5° | 33.0° | 33.5° | 34.0° | 34.5° | 35.0° | |
|---|---|---|---|---|---|---|---|---|---|---|
| *mm* | | | | | | | | | | *inches* |
| 700 | 0.90912 | 0.90620 | 0.90329 | 0.90082 | 0.89735 | 0.89432 | 0.89129 | 0.88821 | 0.88512 | 27.56 |
| 702 | 0.91188 | 0.90894 | 0.90600 | 0.90303 | 0.90006 | 0.89703 | 0.89400 | 0.89091 | 0.88782 | 27.64 |
| 704 | 0.91459 | 0.91165 | 0.90871 | 0.90576 | 0.90282 | 0.89976 | 0.89671 | 0.89362 | 0.89053 | 27.72 |
| 706 | 0.91735 | 0.91441 | 0.91147 | 0.90847 | 0.90547 | 0.90241 | 0.89935 | 0.89627 | 0.89318 | 27.80 |
| 708 | 0.92006 | 0.91712 | 0.91418 | 0.91118 | 0.90818 | 0.90512 | 0.90206 | 0.89897 | 0.89588 | 27.87 |
| 710 | 0.92276 | 0.91982 | 0.91688 | 0.91388 | 0.91088 | 0.90782 | 0.90476 | 0.90168 | 0.89859 | 27.95 |
| 712 | 0.92553 | 0.92256 | 0.91959 | 0.91659 | 0.91359 | 0.91053 | 0.90747 | 0.90438 | 0.90129 | 28.03 |
| 714 | 0.92824 | 0.92529 | 0.92235 | 0.91932 | 0.91629 | 0.91323 | 0.91018 | 0.90706 | 0.90394 | 28.11 |
| 716 | 0.93100 | 0.92803 | 0.92506 | 0.92203 | 0.91900 | 0.91594 | 0.91288 | 0.90976 | 0.90665 | 28.19 |
| 718 | 0.93371 | 0.93078 | 0.92776 | 0.92474 | 0.92171 | 0.91865 | 0.91559 | 0.91247 | 0.90935 | 28.27 |
| 720 | 0.93641 | 0.93344 | 0.93047 | 0.92744 | 0.92441 | 0.92135 | 0.91829 | 0.91517 | 0.91206 | 28.35 |
| 722 | 0.93918 | 0.93618 | 0.93318 | 0.93015 | 0.92712 | 0.92412 | 0.92100 | 0.91785 | 0.91471 | 28.43 |
| 724 | 0.94188 | 0.93897 | 0.93606 | 0.93294 | 0.92982 | 0.92676 | 0.92371 | 0.92056 | 0.91741 | 28.50 |
| 726 | 0.94459 | 0.94159 | 0.93859 | 0.93556 | 0.93253 | 0.92944 | 0.92635 | 0.92323 | 0.92012 | 28.58 |
| 728 | 0.94735 | 0.94435 | 0.94135 | 0.93830 | 0.93544 | 0.93215 | 0.92906 | 0.92591 | 0.92276 | 28.66 |
| 730 | 0.95006 | 0.94706 | 0.94406 | 0.94103 | 0.93800 | 0.93488 | 0.93176 | 0.92861 | 0.92547 | 28.74 |
| 732 | 0.95282 | 0.94979 | 0.94676 | 0.94373 | 0.94071 | 0.93759 | 0.93447 | 0.93132 | 0.92818 | 28.82 |
| 734 | 0.95553 | 0.95250 | 0.94947 | 0.94644 | 0.94341 | 0.94034 | 0.93718 | 0.93403 | 0.93088 | 28.90 |
| 736 | 0.95824 | 0.95521 | 0.95218 | 0.94915 | 0.94612 | 0.94300 | 0.93988 | 0.93670 | 0.93353 | 28.98 |
| 738 | 0.96100 | 0.95797 | 0.95494 | 0.95188 | 0.94882 | 0.94570 | 0.94259 | 0.93941 | 0.93624 | 29.06 |
| 740 | 0.96371 | 0.96068 | 0.95765 | 0.95459 | 0.95153 | 0.94841 | 0.94529 | 0.94211 | 0.93894 | 29.13 |
| 742 | 0.96647 | 0.96341 | 0.96035 | 0.95730 | 0.95424 | 0.95112 | 0.94800 | 0.94482 | 0.94165 | 29.21 |
| 744 | 0.96918 | 0.96615 | 0.96312 | 0.96003 | 0.95694 | 0.95382 | 0.95071 | 0.94750 | 0.94429 | 29.29 |
| 746 | 0.97188 | 0.96885 | 0.96582 | 0.96273 | 0.95965 | 0.95653 | 0.95341 | 0.95020 | 0.94700 | 29.37 |
| 748 | 0.97465 | 0.97159 | 0.96853 | 0.96544 | 0.96235 | 0.95925 | 0.95606 | 0.95288 | 0.94971 | 29.45 |
| 750 | 0.97735 | 0.97429 | 0.97124 | 0.96815 | 0.96506 | 0.96191 | 0.95876 | 0.95558 | 0.95241 | 29.53 |
| 752 | 0.98006 | 0.97703 | 0.97400 | 0.97088 | 0.96776 | 0.96461 | 0.96147 | 0.95826 | 0.95506 | 29.61 |
| 754 | 0.98282 | 0.97976 | 0.97671 | 0.97359 | 0.97047 | 0.96732 | 0.96418 | 0.96097 | 0.95776 | 29.69 |
| 756 | 0.98553 | 0.98247 | 0.97941 | 0.97629 | 0.97318 | 0.97003 | 0.96688 | 0.96367 | 0.96047 | 29.76 |
| 758 | 0.98829 | 0.98521 | 0.98212 | 0.97900 | 0.97588 | 0.97273 | 0.96959 | 0.96638 | 0.96318 | 29.84 |
| 760 | 0.99100 | 0.98794 | 0.98488 | 0.98176 | 0.97865 | 0.97547 | 0.97229 | 0.96908 | 0.96588 | 29.92 |
| 762 | 0.99371 | 0.99065 | 0.98759 | 0.98443 | 0.98135 | 0.97817 | 0.97500 | 0.97176 | 0.96853 | 30.00 |
| 764 | 0.99647 | 0.99338 | 0.99029 | 0.98717 | 0.98406 | 0.98088 | 0.97771 | 0.97447 | 0.97124 | 30.08 |
| 766 | 0.99918 | 0.99609 | 0.99300 | 0.98988 | 0.98676 | 0.98356 | 0.98035 | 0.97714 | 0.97394 | 30.16 |
| 768 | 1.00188 | 0.99880 | 0.99571 | 0.99259 | 0.98947 | 0.98629 | 0.98312 | 0.97986 | 0.97659 | 30.24 |
| 770 | 1.00465 | 1.00156 | 0.99847 | 0.99532 | 0.99218 | 0.98897 | 0.98576 | 0.98252 | 0.97929 | 30.31 |
| °F | 87.8° | 88.7° | 89.6° | 90.5° | 91.4° | 92.3° | 93.2° | 94.1° | 95.0° | |

*Various strength solutions of the common acids, alkalies, and alcohol.*[1] 26

(a) Hydrochloric Acid Solutions: Specification requires not less than 35% HCl by weight. Sp. gr. = 1.1778 at 15°. Mix with water and make up to 1 liter.

| HCl STRENGTH DESIRED GRAMS PER LITER | HYDROCHLORIC ACID REQUIRED | | |
|---|---|---|---|
| | g | cc | |
| 5 | 14.28 | 12.13 | |
| 10 | 28.57 | 24.26 | |
| 15 | 42.85 | 36.39 | |
| 20.24 | 57.82 | 49.10 | Constant boiling |
| 36.46 | 104.17 | 88.43 | Normal solution |
| 50 | 142.85 | 121.30 | |
| 100 | 285.71 | 242.60 | |
| 150 | 428.57 | 363.90 | |
| 200 | 571.43 | 485.20 | |
| 247.5 | 707.14 | 600.40 | Sp. gr. 1.125 |
| 300 | 857.14 | 727.80 | |

(b) Sulfuric Acid Solutions: Specification requires not less than 94% $H_2SO_4$ by weight. Sp. Gr. = 1.835 at 15°. Pour acid into excess of water and make up to 1 liter.

| $H_2SO_4$ STRENGTH DESIRED GRAMS PER LITER | SULFURIC ACID REQUIRED | | |
|---|---|---|---|
| | g | cc | |
| 5 | 5.32 | 3.0 | |
| 12.5 | 13.29 | 7.2 | For crude fiber |
| 20 | 21.28 | 11.6 | |
| 30 | 31.91 | 17.4 | |
| 40 | 42.55 | 23.2 | |
| 49 | 52.13 | 28.4 | Normal solution |
| 100 | 106.38 | 58.0 | |
| 150 | 159.57 | 87.0 | |
| 250 | 265.96 | 144.9 | |
| 300 | 319.15 | 173.9 | |
| 400 | 425.53 | 231.9 | |

(c) Nitric Acid Solutions: Specification requires not less than 68% $HNO_3$ by weight. Sp. gr. = 1.4146 at 15°. 1 cc of concentrated $HNO_3$ contains approximately 0.96 g of $HNO_3$. Mix with water and make up to 1 liter.

| $HNO_3$ STRENGTH DESIRED GRAMS PER LITER | NITRIC ACID REQUIRED | |
|---|---|---|
| | g | cc |
| 5 | 7.35 | 5.2 |
| 10 | 14.71 | 10.4 |
| 20 | 29.41 | 20.8 |
| 30 | 44.12 | 31.2 |
| 40 | 58.82 | 41.6 |
| 50 | 73.53 | 52.0 |
| 63 | 92.65 | 65.5 |
| 70 | 102.94 | 72.8 |
| 100 | 147.06 | 104.0 |
| 150 | 220.59 | 156.0 |
| 200 | 294.12 | 207.9 |
| 300 | 441.18 | 312.9 |

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[1] Prepared by G. C. Spencer.

(d) Ammonia Solutions: Specification requires not less than 27% $NH_3$ by weight. Sp. gr. = 0.9. Mix and make to 1 liter.

| $NH_3$ STRENGTH DESIRED GRAMS PER LITER | REAGENT AMMONIA REQUIRED | |
|---|---|---|
| | g | cc |
| 5 | 18.52 | 20.6 |
| 10 | 37.04 | 41.1 |
| 15 | 55.55 | 61.7 |
| 20 | 74.07 | 82.3 |
| 25 | 92.59 | 102.9 |
| 50 | 185.18 | 205.8 |
| 75 | 277.77 | 308.6 |
| 100 | 370.37 | 411.5 |
| 150 | 555.55 | 617.3 |
| 200 | 740.74 | 823.0 |

(e) Sodium Hydroxide Solutions: Specification requires 95% NaOH in sticks of caustic soda. Dissolve and dilute to 1 liter.

| NaOH STRENGTH DESIRED GRAMS PER LITER | SODIUM HYDROXIDE REQUIRED | |
|---|---|---|
| | g | |
| 12.5 | 13.16 | For crude fiber |
| 30 | 31.58 | |
| 40 | 42.11 | Normal solution |
| 50 | 52.63 | |
| 75 | 78.95 | |
| 100 | 105.26 | |
| 150 | 157.89 | |
| 200 | 210.53 | |
| 250 | 263.16 | |
| 300 | 315.79 | |

(f) Alcoholic Solutions[1]: Specification requires 95% $C_2H_5OH$ by volume. Sp.gr. = 0.810 at 25°. Mix and make to 1 liter.

| ALCOHOL STRENGTH DESIRED CC PER LITER | ALCOHOL REQUIRED | |
|---|---|---|
| | cc | g |
| 50 | 52.6 | 42.63 |
| 100 | 105.3 | 85.26 |
| 150 | 157.9 | 127.89 |
| 200 | 210.5 | 170.52 |
| 250 | 263.2 | 213.16 |
| 300 | 315.9 | 255.78 |
| 400 | 421.1 | 341.04 |
| 500 | 526.3 | 426.32 (proof) |
| 700 | 736.8 | 596.84 |

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[1] Alcohol of any desired strength may be obtained by taking the number of cc of 95% alcohol equivalent to the desired strength and making the solution up to 95 cc. For example.—To obtain a solution of 70% alcohol, take 70 cc of 95% alcohol and dilute to 95 cc.

# INDEX

PAGE

Abbé refractometer, index of refraction of oils, fats and waxes.............. 316
Abrastol (asaprol).............................................. 346
Acetanilid..................................................... 437–442
Acetic acid in acetylsalicylic acid.................................... 447
Acetic acid test, critical temperature of dissolution....................... 160
Acetone-carbon tetrachloride test, paraffin, tallow, etc., in chocolate products. 161
Acetphenetidin...............................437, 444, 445, 448
Acetyl value, fats, oils and waxes..................................... 326
Acetylsalicylic acid..................................445, 447, 448
Acid, in honey.................................................. 389
    salad dressings............................................. 355
  potassium tartrate in fruits and fruit products.......................... 272
  standard solution............................................... 16
Acidity, in beverages and concentrates................................. 131
      cheese.................................................. 239
      coffee.................................................. 152
      distilled liquors.......................................... 143
    of fat, eggs and egg products.................................... 248
      fruits and fruit products................................... 270
      milk.................................................... 214
      prepared mustard.......................................... 354
      tanning material liquors.................................... 99
      water extract of wheat flour................................. 167
    water-soluble, in fruits and fruit products.......................... 285
Acids, carbonic and bicarbonic, in mineral water.......................... 412
  free mineral, in fruits and fruit products.............................. 274
  in beers........................................................ 135
    fats and oils................................................. 322
    tomato products.............................................. 399
    vegetables and vegetable products.............................. 398
    vinegars...................................................... 357
    wines........................................................ 140
  various strength solutions.......................................... 563
  volatile, in beverages and concentrates............................... 131
        fruits and fruit products.................................... 270
Acid-soluble boric acid in fertilizers................................... 29
Agar agar in fruits and fruit products.................................. 275
Agricultural liming materials.......................................... 32
Albumin in milk................................................. 215
      unsweetened evaporated milk.................................. 228
      wheat flour................................................ 168
Alcohol, ethyl....................................................476, 477
    extract in spices and other condiments.............................. 350
    in almond extract............................................. 259

PAGE
- beers........ 134
- beverages and concentrates........ 131
- cassia, cinnamon and clove extracts........ 260
- distilled liquors........ 143
- fruits and fruit products........ 264
- ginger extracts........ 261
- lemon and orange extracts........ 255
- peppermint, spearmint and wintergreen extracts........ 261
- sirups used in confectionery........ 387
- vanilla extract........ 251
- vinegars........ 358
- wines........ 136
- methyl........ 476
- various strength solutions........ 563

Alcohol precipitate of fruits and fruit products........ 269
Alcohol tables........ 532
Alcoholic potassium hydroxide........ 321
Aldehydes in distilled liquors........ 144
- lemon and orange extracts and oils........ 256, 258

Alimentary pastes........ 180
Alkali salts, in soils........ 13
Alkalies, in industrial water........ 422
- various strength solutions........ 563

Alkaline salt solution........ 462
Alkalinity of ash, beverages and concentrates........ 131
- cacao products........ 157
- confectionery........ 385
- fruits and fruit products........ 266
- maple products........ 392
- roasted coffee........ 151
- sugars and sugar products........ 366
- tea........ 154
- tomato products........ 399
- vinegars........ 357
- wines........ 139
- industrial water........ 423

Alkaloids, cinchona........ 452
- microchemical tests........ 450, 451
- opium........ 461

Allihn's gravimetric method, dextrose........ 384
- table, determination of dextrose........ 524

Almond extract........ 259
Alpha-naphthylamine hydrochloride reagent........ 405
Alumina cream........ 368
Alumina in paints........ 87
Aluminum, in baking powders and baking chemicals........ 122, 123
- commercial coal tar food colors........ 202
- industrial water........ 422
- mineral water........ 414
- plants........ 103

PAGE
Aluminum oxides in plants 103
  soils 6
Aluminum wire reagent 459
Amaranth, isomeric and similar dyes content 210
Amidopyrin 470
Amino nitrogen in meat and meat products 297
  wheat flour 168
Ammonia, in baking powders and baking chemicals 124
  meat and meat extracts 290, 301
Ammoniacal nitrogen in fertilizers 22
Ammonium chloride solution 25
Ammonium citrate solution 17
Ammonium tartrate solution 271
Ampuls, sampling 437
Anise and nutmeg extracts 262
Anthranilic acid ester in beverages and concentrates 132
Antimony oxide in paints 86
Antipyrin 449
Apomorphine in tablets 463
Appendix 490
Arecoline, threshold myotic dose 486
Arsenic, in arsphenamine and neoarsphenamine 479
  baking powders and baking chemicals 130
  Bordeaux mixtures 49–51
  calcium arsenate 45
  commercial coal tar food colors 203
  foods 306
  gelatin 303
  insecticides and fungicides 35, 38
  iron-arsenic tablets 477
  iron methyl arsenate 478
  lead arsenate 43
  London purple 42
  magnesium arsenate 47
  mineral water 420
  Paris green 40
  plants 109
  sodium cacodylate 478
  zinc arsenite 47
Arsenic oxide in lead arsenate 44
  London purple 42
Arsenicals 477
Arsenious oxide, in Bordeaux mixture with Paris green 49
  calcium arsenate 45
  lead arsenate 44
  London purple 42
  magnesium arsenate 47
  Paris green 40
  zinc arsenite 47
  standard solution 35, 420

PAGE

- Arsphenamine ... 479
- Artificial sweeteners ... 334
- Asaprol ... 346
- Asbestos ... 280
- Ascaridole in oil of chenopodium ... 485
- Ash, in alimentary pastes ... 181
    - baking powders and baking chemicals ... 123
    - beers ... 135
    - beverages and concentrates ... 131
    - bread ... 178
    - cacao products ... 157
    - coffee ... 150
    - commercial coal tar food colors ... 200
    - confectionery ... 385
    - dairy products ... 215, 225, 227, 228, 230, 237, 239
    - distilled liquors ... 143
    - fruits and fruit products ... 265
    - gelatin ... 303
    - grain and stock feeds ... 278
    - honey ... 388
    - leathers ... 93
    - lemon and orange extracts ... 257
    - maple products ... 392
    - meat and meat products ... 289, 300
    - plants ... 102
    - prepared mustard ... 354
    - soap emulsions ... 55
    - spices and other condiments ... 349
    - sugars and sugar products ... 365
    - tea ... 154
    - vanilla extract ... 253
    - vegetables and vegetable products ... 397, 399
    - vinegars ... 357
    - wheat flour ... 166
    - wines ... 139
  - of fruits and fruit products, sulfur content ... 266
- Ash-extract ratio of wines ... 139
- Atropine, in tablets ... 452
    - microchemical identification ... 451
    - threshold mydriatic dose ... 486

- Babcock method, fat, dairy products ... 217, 225
- Bacteria in tomato products ... 401
- Bailey-Andrew method, caffeine in tea ... 155
- Baked cereal products ... 177
- Baking powders and baking chemicals ... 115
- Baking test, experimental, for bread ... 178
- Barbital ... 484
- Barium in mineral water ... 417
    - paints ... 86

PAGE
Basic lead nitrate 369
Basic slag 27
Baudouin test, sesame oil 331
Beers 134
Beets, sugar 394
Benedict's solutions 378
Benzaldehyde in almond extract 259
Benzoic acid 336
 in almond extract 259
Beta-naphthol 345
Beverages (non-alcoholic) and concentrates 131
Bioassay of drugs 485
Black alkali in irrigation water 424
Bone and tankage, mechanical analysis 14
Bone in meat scrap or tankage 287
Borates 339
Bordeaux mixtures 48–50
Boric acid, in fertilizers 28
 mineral water 420
Boric acid and borates 339
Bread 177
 experimental baking text 178
 total solids in entire loaf 177
Brine 421, 424
Bromate standard solution 35
Bromcresol purple indicator 473
Bromthymol blue indicator 56
Bromide, in brine 426
 mineral water 419
Brucine, microchemical identification 451
Butter 231
 renovated 238

Cacao bean and its products 157
Cacao shell, detection in cacao products 162
Caffeine 439, 441, 442, 444, 448, 449
 in coffee 151
 tea 155
 microchemical identification 451
Calcium, in baking powders and baking chemicals 123
 commercial coal tar food colors 202
 fruits and fruit products 268
 industrial water 422
 mineral water 414
 paints 88
 plants 104
 salt 429
 soils 6
Calcium arsenate 45, 46
Calcium chloride 116

PAGE

Calcium cyanide 52
Calcium oxide, in agricultural liming materials 34
  ash, spices 349
  mineral feeds 287
Calomel 481
Camphor 458
Canadian lead number, maple products 393
Cane sugar, densities of solutions 506
Capsicum in ginger extract 261
Capsules, soft, sampling 437
Caramel in distilled liquors 148
Carbohydrates in plants 112
Carbon, carbonate, in soils 2, 4
Carbon dioxide, corresponding factors for gasometric determination 558
  in agricultural liming materials 34
    baking powders and baking chemicals 115, 120
    Bordeaux mixtures 48–50
    mineral water 412
    paints 89
Carbon tetrachloride 145, 479
Carbonate carbon in soils 2
Carbonate hydroxide in soda lye 55
Carbonic and bicarbonic acids in industrial water 422
  mineral water 412
Casein, in butter 237
  milk 215
  milk chocolate 157
  unsweetened evaporated milk 228
Cassia 260
Cat-eye method for mydriatics and myotics 485
Caustic poisons 66
Caustic value of agricultural liming materials 32
Cereal foods 165
Cereal products, baked 177
Chaulmoogra oil 484
Cheese 238
Chenopodium oil 484
Chicory infusion is coffee 153
Chloride in industrial water 422
  mineral water 412
  potable water 406
Chlorides in ash, alimentary pastes 181
  bread 178
  meat extracts 301
  roasted coffee 151
  wines 139
Chlorine in ash, calcium cyanide 53
  eggs and egg products 249
  fertilizers 30
  fruits and fruit products 266

PAGE

Chlorine in plants........ 111
    sodium and potassium cyanides........ 51
    wheat flour........ 173
Chloroform........479–481
Chocolate, milk, casein content........ 157
Chocolate extract........ 260
Cholesterol and phytosterol in mixtures of animal and vegetable fats........ 327
Chromium trioxide in sulfuric acid, insecticides and fungicides........ 60
Cinchona alkaloids........ 452
Cinchonidine........ 451
Cinchonine........ 451
Cinnamon extract........ 260
Citral in orange and lemon extracts........ 257
Citric acid, in beverages and concentrates........ 131
    cheese........ 241
    fruits and fruit products........ 273
Clarifying reagents for sucrose........ 368
Clove extract........ 260
Coal tar dyes........184, 207
Coal tar food colors........187, 197
Coating and glazing substances, coffee........ 153
Cocaine........451, 474
    threshold mydriatic dose........ 486
Cochineal indicator........ 19
Coconut oil in cacao butter and fat extracted from chocolate........ 159
Codeine........441, 451
    in tablets........ 463
Coffee and tea........ 150
Cold test for oils, fats and waxes........ 331
Cold water extract, spices and other condiments........ 350
Color, added, alimentary pastes........ 181
    eggs and egg products........ 249
    in beers........ 134
        distilled liquors........ 147
        vinegars........ 360
    potable water........ 403
    turpentine oil........ 68
Color acid and dye in commercial coal tar food colors........ 205
Color reactions produced on dyed fibers by various reagents........ 190
Color value, gasoline, wheat flour........ 176
    of vanilla extracts........ 254
Coloring matters, in beers........ 136
    beverages and concentrates........ 132
    butter........ 237
    cacao products........ 164
    cheese........239, 243
    confectionery........ 387
    cream........ 227
    distilled liquors........ 147
    foods........ 184

PAGE
        fruits and fruit products ... 276
        green coffee ... 150
        lemon and orange extracts ... 257
        meat ... 295
        milk ... 224
        oils, fats and waxes ... 332
        prepared mustard ... 354
        unsweetened evaporated milk ... 228
        vegetables and vegetable products ... 398
        vinegars ... 362
        wines ... 141, 142
      natural ... 194
      other than caramel in vanilla extracts ... 255
Colors, artificial, Marsh's test ... 148
    food, commercial coal tar ... 197
    in amyl alcohol ... 148
      vanilla extract ... 254
Commercial coal tar food colors ... 197
Commercial glucose ... 376
        in confectionery ... 385
          fruits and fruit products ... 275
          honey ... 389
          maple products ... 392
Commercial invert sugar, honey ... 390
Condensed milk (sweetened) ... 228
Condiments ... 349
Conductivity value, maple products ... 393
Confectionery ... 385
Copper, in Bordeaux mixtures ... 49, 50, 51
      food ... 311
      gelatin ... 303
      insecticides and fungicides ... 39
      plants ... 107, 113
      potable water ... 410
      standard solution ... 410
Copper carbonate ... 48
Copper oxide, in insecticides and fungicides ... 41, 48
Copper sulfate, standard solution ... 107
Copper-reducing substances, spices and other condiments ... 350, 354
Corn, field, in mixtures of field and sweet corn ... 401
Correction for variation of turpentine in atmospheric pressure ... 71
Cottonseed oil in oils, fats and waxes ... 330
Coumarin in vanilla extract ... 251
Cowles malic acid value, maple products ... 394
Cream ... 224
Cream of tartar in wines ... 141
Creatin in meat and meat products ... 296, 302
Creatinin in meat extracts ... 302
Critical temperature of dissolution in acetic acid test ... 160
Crude fiber, in alimentary pastes ... 181

PAGE
Crude fiber, in bread .......... 178
    cacao products .......... 163
    coffee .......... 152
    grain and stock feeds .......... 280
    plants .......... 114
    prepared mustard .......... 354
    spices and other condiments .......... 350
    tea .......... 155
    wheat flour .......... 167
Crude protein in wines .......... 142
Cryoscopic method, added water in milk .......... 219
Cuprous oxide, precipitation .......... 113, 379
Cyanide, calcium .......... 52
Cyanides, sodium and potassium .......... 51
Cyanogen .......... 51, 52

Dairy products .......... 214
Decomposition in eggs and egg products, detection .......... 248
Devarda alloy, reagent .......... 459
  method, nitrogen in nitrate salts .......... 23
Dextrin, in beers .......... 135
    coffee .......... 153
    fruits and fruit products .......... 275
    honey .......... 389
    vinegars .......... 362
    wines .......... 142
Dextrose .......... 384
  in honey .......... 389
Diacetylmorphine, in tablets .......... 464
    microchemical identification .......... 451
Diastase in honey .......... 390
Diammonium citrate solution .......... 271
Dichlorhydrin .......... 457
Dipotassium phosphate .......... 94
Disodiumphosphate solution .......... 450
Distilled liquors .......... 143
Domke's table, apparent specific gravity of sucrose solutions, 20°C .......... 504
Dried milk .......... 229
Drugs .......... 437
  bioassay .......... 485
  sampling .......... 437
Dulcin .......... 346
Dust, stems and foreign leaves in tea .......... 154
Dyes, acid .......... 186, 187

Edestin in wheat flour .......... 168
Egg, whole or commercial yolk, detection .......... 182, 249
Egg albumin and gelatin in glazing of coffee .......... 153
Egg solids, estimation .......... 249
    in alimentary pastes .......... 182
      salad dressing .......... 356

PAGE

Eggs and egg products.......... 244
Electroscope, standardization, radioactivity in food and drugs.......... 434
Emetine hydrochloride.......... 450
Emulsions, mineral oil—soap.......... 54
Ephedra.......... 454
Ephedrine,..........451–456
    threshold mydriatic dose.......... 486
Ergot, assay.......... 487
Eserine.......... 486
Esters, in beverages and concentrates..........131
    distilled liquors.......... 144
Ether extract, in confectionery.......... 386
    grain and stock feeds.......... 279
    meat.......... 290
    plants.......... 114
    prepared mustard.......... 354
    spices and other condiments.......... 349
    wheat flour.......... 166
Ether extractives in commercial coal tar food colors.......... 204
Ethyl alcohol.......... 476
Euphthalmin, threshold mydriatic dose.......... 486
Evaporated milk (unsweetened).......... 227
Experimental baking test for bread.......... 178
Extract, in beers.......... 134
    distilled liquors.......... 143
    tanning materials.......... 101
    wheat flour.......... 172
    wines.......... 138
Extraction, of added color.......... 181
    raw and spent materials, tanning materials.......... 100
    water-soluble material, leathers.......... 93
Extracts. *See* Flavoring extracts.......... 251

Facing for tea.......... 156
Fat, acidity of, eggs and egg products.......... 248
  extracted from milk chocolate, examination.......... 160
  in alimentary pastes.......... 181
    bread.......... 178
    butter.......... 236
    cacao products.......... 158
    cheese..........239, 243
    cream.......... 225
    dried milk.......... 230
    eggs and egg products.......... 246
    grain and stock feeds.......... 279
    malted milk.......... 230
    meat and meat products..........290, 301
    milk.......... 216
    sweetened condensed milk.......... 228
    unsweetened evaporated milk.......... 227

PAGE
Fat, in wheat flour........ 166
Fats........ 314
    and waxes in coffee........ 153
Fatty acids in fats, oils and waxes........324, 326
    paints........77, 78
Fendler-Stüber method (modified), caffeine in coffee........ 151
Fermentation in beers........ 135
Ferric and aluminum oxides in plants........ 103
Ferric sulfate, standard solution........ 75
Fertilizers........ 14
Fertilizers and liming materials, definitions of terms and interpretation of results 490
Field corn in canned mixtures of field and sweet corn........ 401
Filtering cloth........ 280
Fish oil and marine animal oils........ 332
Flavoring extracts........ 251
Flour, wheat........ 165
Fluorides in baking powder and baking chemicals........ 126
    preservatives and artificial sweeteners........ 342
Fluorine in foods........ 312
    insecticides and fungicides........ 59
Food colors, permitted coal tar........ 187
Food, radioactivity........ 431
Foods, coloring matter content........ 184
    metals content........ 306
Formaldehyde........ 340
Formaldehyde solutions........ 56
Formic acid........ 346
    in vinegars........ 361
Fowler modification (Canadian lead number) maple products........ 393
Fruits and fruit products........ 264
Fuchsin-sulfurous acid mixture........ 476
Fungicides and insecticides........ 35
Furfural in distilled liquors........ 144
Fusel oil in distilled liquors........ 145

Galactan in grain and stock feeds........ 285
Gasoline color value, wheat flour........ 176
Gelatin in cream........ 227
    fruits and fruit products........ 275
    glazing of coffee........ 153
    meat extracts........301, 303
    milk........ 223
    unsweetened evaporated milk........ 228
Gelatin nitrogen in meat........ 296
Gelatin solution, tanning materials........ 99
Ginger extract........ 261
Glazing substances for coffee........ 153
Globulin and albumin and amino nitrogen in wheat flour........ 168
Glucose, in beverages and concentrates........ 131
    fruits and fruit products........ 275

PAGE

Glucose, in honey . . . 390
  leathers . . . 94
  sugars and sugar products . . . 376
  wines . . . 139
Glucosides in beans . . . 287
Gluten in wheat flour . . . 169
Glutenin in wheat flour . . . 168
Glycerol, in beers . . . 135
  lemon and orange extracts . . . 255
  meat and meat extracts . . . 293, 302
  vanilla extract . . . 251
  vinegars . . . 358
  wines . . . 137
Glycerol-alcohol ratio, wines . . . 138
Glycogen in meat and meat products . . . 293
Grain and stock feeds . . . 277
Grape juice, detection of added water . . . 276
Grit in poultry and similar feeds . . . 286
Gum and dextrin in wines . . . 142
Gunning methods . . . 20, 21
Gunning-Hibbard method, total nitrogen in soils . . . 5
Gutzeit method, arsenic in foods . . . 306

Halogens in commercial coal tar food colors . . . 211
Halphen test, cottonseed oil in oils, fats and waxes . . . 330
Halphen-Hicks test, rosin in paints . . . 78
Hanus method, iodine absorption number . . . 319
Hardness of industrial water . . . 422
Heavy metals in foods . . . 309
Hehner number, fats and oils . . . 322
Hehner test, formaldehyde . . . 341
Heidenhain's apparatus, carbon dioxide . . . 116
Hematin in tanning materials . . . 99
Heroin in tablets . . . 464
  microchemical identification . . . 451
Herzfeld's methods . . . 381
  table, invert sugar in raw sugars . . . 520
Hexamethylenetetramine . . . 456
Hide powder . . . 97
Hide substance . . . 95
Hiltner method, total aldehydes in lemon and orange oils . . . 258
Homatropine, threshold mydriatic dose . . . 486
Honey . . . 387
Hortvet cryoscope . . . 219
Hübener's table . . . 513
Hydrochloric acid, determination of strength . . . 18
  —stannous chloride solution . . . 306
Hydrocyanic acid, in almond extract . . . 260
  beans . . . 287
Hydrogen peroxide reagent . . . 56

PAGE

Hydrogen sulfide in mineral water.... 412
Hydrogen-ion concentration, soils.... 12
  wheat flour.... 167
Hydroxide and carbonate in soda lye.... 55
Hyoscyamine, threshold mydriatic dose.... 486

Ice cream.... 242
Identification of natural coloring matters.... 194
  permitted coal tar food colors.... 188
Index of refraction of cacao fat.... 161
  lemon and orange oils.... 258
  oils, fats and waxes.... 315
  turpentine oil.... 68
Indian gum.... 476
Indigo solution.... 142
Industrial water.... 422
Insecticides and fungicides.... 35
International atomic weights.... 496
International Commission for Unifying Methods of Sugar Analysis, rules.... 367
Invert sugar.... 377
Iodide, in brine.... 424
  mineral water.... 419
  mercuric, in tablets.... 461
Iodine, in soils.... 8
  organically combined in commercial coal tar food colors.... 210
Iodine number of cacao fat.... 161
  fatty acids, paints.... 78
  oils, fats and waxes.... 319
  paprika oil.... 351
Iodine solution.... 35, 75, 412
Iodoform.... 483
Ionizable silver compounds.... 483
Ipecac, fluidextract.... 475
Iron, in baking powders and baking chemicals.... 123
  commercial coal tar food colors.... 202
  mineral and industrial water.... 413, 422
  plants, micro method.... 103
  methylarsenates, arsenate content.... 478
  oxide of, in soils.... 6
Irrigating water.... 424
Isomeric and similar dyes, in amaranth.... 210

Jones' modification of Robertson method, nitrate nitrogen in fertilizers.... 23
Jorissen method, sucrol or dulcin.... 346
  test, salicylic acid.... 335

Kaolin, tanning materials.... 99
Kerr-Sorber method, unsaponifiable residue in alimentary pastes.... 181
  wheat flour.... 171
Kirschner value, fats and oils.... 324

PAGE

Kjeldahl method, modified to include the nitrogen of nitrates ... 21
    total nitrogen in soils ... 5
Kjeldahl-Gunning-Arnold method, organic and ammoniacal nitrogen in fertilizers ... 21
Kleber method, total aldehydes in lemon and orange oils ... 258
Knorr's apparatus, carbon dioxide ... 115
Koettstorfer number, fats and oils ... 321
Kraut's reagent ... 450
Kröber's table, pentoses and pentosans ... 529

Lactose ... 383
    in cacao products ... 158
        cream ... 225
        milk ... 216
        sweetened condensed milk ... 228
        unsweetened evaporated milk ... 228
Lane-Eynon method, invert sugar ... 377
Langer's method, santonin ... 472
Lard, detection of foreign fats containing tristearin ... 331
Leach test for formaldehyde ... 341
Lead, antimony, paints ... 84
    basic carbonate of, paints ... 79
    basic sulfate of, paints ... 80
    in baking powders and baking chemicals ... 124
        commercial coal tar food colors ... 201
        foods ... 312
        potable waters ... 409
Lead acetate cotton ... 306
Lead arsenate ... 43
Lead number, maple products ... 392
    vanila extract ... 252
Lead oxide in Bordeaux mixture ... 50
    insecticides and fungicides ... 39
    lead arsenate ... 45
    standard solution ... 409
Leathers ... 92
Lecithin-phosphoric acid ($P_2O_5$) in salad dressings ... 356
Leffman-Beam method, soluble volatile acids in fats and oils ... 324
Lemon and orange extracts and oils ... 255, 257
Lemon and orange peel color, detection ... 257
Lemon oil in vegetable and mineral oils ... 262
Leucosin in wheat flour ... 168
Levant wormseed ... 472
Levulose in honey ... 388
Liebermann-Storch test, rosin in paints ... 78
Lime, in lime-sulfur solutions ... 59
Lime oil in vegetable and mineral oils ... 262
Limestone, ground, mechanical analysis ... 34
Lime-sulfur solutions ... 57
Liming materials, agricultural ... 32

PAGE
Liming materials, definition of terms and interpretation of results........... 490
Lindo-Gladding method, potash in fertilizers.............................. 25
Linseed oil paints, white.................................................. 73
Lipoid phosphoric acid ($P_2O_5$)........................................ 247
    in alimentary pastes..................... 181
    wheat flour.............................. 170
Lipoids in eggs and egg products........................................... 247
    wheat flour.............................................................. 170
Liquors, distilled.....................................................134, 143
    in tanning materials.................................................... 98
Lithium in mineral water................................................... 415
Lithopone in paints........................................................ 81
Litmus indicator........................................................... 56
Locke-Ringer solution, modified............................................ 487
Logwood method, free mineral acids in vinegars............................ 361
London purple.............................................................. 42
Loss on ignition, soils.................................................... 2
Lye, soda.................................................................. 55

McGill method, modified, starch............................................ 122
Macroscopic examination of coffee.......................................... 150
Magnesia mixture........................................................... 14
Magnesium in commercial coal tar food colors............................... 202
    fruits and fruit products............................................... 269
    industrial water........................................................ 422
    mineral water........................................................... 415
    paints.................................................................. 88
    plants.................................................................. 106
    salt.................................................................... 429
    soils................................................................... 7
Magnesium arsenate......................................................... 47
Magnesium oxide, in agricultural liming materials.......................... 34
Malic-acid value, maple products........................................... 394
Malt extract............................................................... 282
Malted milk................................................................ 229
Maltose.................................................................... 383
Manganese in foods......................................................... 313
    fruits and fruit products............................................... 267
    mineral water........................................................... 418
    plants.................................................................. 106
    soils................................................................... 6, 7
Manganous sulfate, standard solution....................................8, 418
Maple products and maple sirup............................................. 391
Marme's reagent............................................................ 450
Marsh's test, artificial colors in distilled liquors........................ 148
Martius yellow in napthol yellow S......................................... 211
Massecuites................................................................ 363
Matter soluble in water, paints............................................ 90
Mayer's reagent............................................................ 352
Meat and meat products..................................................... 289

PAGE

Meat bases........ 296
Meat extracts and similar products........ 300
Meat scrap, bone content........ 287
Mechanical analysis of bone and tankage........ 14
Meissl and Hiller's factors, invert sugar........ 521
Melting point of cacao fat........ 161
  commercial coal tar food colors........ 208
  fats and fatty acids........ 317
Menthol........ 468
Mercuric chloride solution, reagent from alkaloids........ 450
Mercuric iodide in tablets........ 461
Mercurous chloride in tablets........ 481
Mercurous iodide........ 482
Mercury in organic mercurial seed disinfectants........ 62
Mercury-thiocyanate reagent, insecticides and fungicides........ 40
Metals in beers........ 136
  beverages and concentrates........ 132
  commercial coal tar food colors........ 201
  confectionery........ 387
  foods........ 306
  fruits and fruit products........ 276
  maple products........ 394
  meat and meat extracts........ 295, 303
  vegetables and vegetable products........ 398
  vinegars........ 362
Methanol, detection in alcoholic beverages........ 148
Methenamine in tablets........ 456
Methyl alcohol........ 476
  in distilled liquors........ 146
    lemon and orange extracts........ 257
    presence of ethyl alcohol........ 476
    vanilla extract........ 254
Methyl anthranilate, standard solution........ 132
Methyl orange indicator, total arsenic........ 36
Methyl red indicator........ 19, 28
Methyl salicylate in wintergreen extract........ 262
Methylene blue........ 456
Methylthionine chloride........ 456
Micro method for iron only in plants........ 103
Microanalysis of tomato pulp, purée, sauce, paste........ 400
Microchemical tests for alkaloids........ 450, 451
Microscopic examination, butter........ 237
  malted milk and its flavored products........ 230
  spices........ 351
Milk........ 214
  chocolate, examination of fat extracted........ 160
  dried........ 229
  fat, in milk chocolate........ 159
  malted........ 229
sweetened condensed........ 228

PAGE
Millon's reagent.....66, 450
Mineral acidity, leathers..... 93
Mineral acids in vinegars..... 361
Mineral adulterants in ash of sugars and sugar products..... 366
Mineral feeds, calcium oxide content..... 287
Mineral oil in paints..... 78
  turpentine oil..... 71
Mineral oil—soap emulsions..... 54
Mineral oils..... 53
Mineral water..... 411
  method of reporting results..... 421
Mohler test, modified, benzoic acid..... 336
Moisture in alimentary pastes..... 180
  Bordeaux mixtures.....48–50
  butter..... 236
  cacao products..... 157
  calcium arsenate..... 45
  cheese..... 238
  commercial coal tar food colors..... 198
  confectionery..... 385
  dried and malted milk..... 229
  dried apples..... 265
  dried fruits..... 264
  fertilizers..... 14
  gelatin..... 303
  grain and stock feeds..... 277
  honey..... 387
  insecticides and fungicides..... 35
  lead arsenate..... 43
  leathers..... 92
  London purple..... 42
  magnesium arsenate..... 47
  maple products..... 391
  meat and meat products..... 289
  Paris green..... 40
  pigments..... 83
  plants..... 102
  raw and spent materials..... 99
  roasted coffee..... 150
  salt..... 428
  soap..... 53
  soils..... 1
  spices and other condiments..... 349
  sugars and sugar products..... 363
  tea..... 154
  vegetables and vegetable products..... 397
  wheat flour..... 165
  zinc arsenate..... 47
Molasses..... 363
Molds in tomato products..... 400

PAGE
Molybdate solution........14, 16
Monier-Williams method, free sulfurous acid in preservatives and artificial sweeteners........ 344
Monobromated camphor........ 58
Monosulfide equivalent, lime sulfur solutions........ 58
Morphine........ 442
    in tablets........ 461
    microchemical identification........ 451
    quantitative tests........ 461
Morpurgo method, sucrol or dulcin........ 346
Munson and Walker method, invert sugar........ 379
    reducing sugars in plants........ 112
    table........ 514
Mustard, prepared........ 353
Mustard seed, volatile oil content........ 351
Mutarotation of sugars and sugar products........ 369
Mydriatics........ 485
Myotics........ 485

Natural coloring matters........ 194
Naval stores........ 68
Neoarsphenamine........ 479
Nessler's reagent........404, 456
Neutral lead acetate........ 369
Neutralizing value of agricultural liming materials........ 32
    baking powders and baking chemicals........ 120
Nicotine in tobacco and tobacco products........ 55
Nitrate nitrogen in fertilizers........ 22
    soils........ 12
Nitrate salts, nitrogen content........ 23
Nitrates, in fertilizers........ 18
    industrial water........ 422
    meat........ 291
    meat extracts........ 302
    potable water........ 405
    wines........ 142
Nitrite, standard solution........ 405
Nitrite nitrogen in wheat flour........ 175
Nitrites in meat........291, 292
    meat extracts........ 302
    potable water........ 405
Nitrobenzol in almond extract........ 260
Nitrogen activity methods for fertilizers........ 23
Nitrogen in alimentary pastes........ 181
    bread........ 178
    cacao products........ 157
    cheese........239, 242
    commercial coal tar food colors........ 211
    confectionery........ 385
    cream........ 225

PAGE
Nitrogen in eggs and egg products ... 245
    fertilizers ... 18
    gelatin ... 303
    grain and stock feeds ... 279
    leathers ... 95
    meat and meat extracts ... 290, 297, 301
    milk ... 215
    mineral water ... 411
    nitrate salts ... 23
    permitted food dyes ... 212
    plants ... 112
    potable waters ... 404
    spices and other condiments ... 349
    soils ... 5, 12
    sugars and sugar products ... 367
    unsweetened evaporated milk ... 227
    wheat flour ... 168, 175
Nitroglycerin ... 459
Nontannins, in tanning material extracts and liquors ... 97, 99
Non-volatile vehicle in paints ... 77
Novocaine ... 470
Nutmeg extract ... 262

Oat hulls in oats and oat feeds ... 286
Odor of potable water ... 403
Oil, in anise and nutmeg extracts ... 262
    cassia cinnamon and clove extracts ... 260
    emulsions ... 54
    peppermint, spearmint and wintergreen extracts ... 261
    salad dressings ... 356
    tea ... 155
Oils, fats and waxes ... 314
    orange and lemon, in extracts ... 255
Oleomargarine ... 238
Opium alkaloids ... 461
Optical rotation of lemon and orange oils ... 258
Orange extracts ... 255
Orange oils ... 257
    in extracts ... 255
        vegetable and mineral oils ... 262
Orange peel color, detection ... 257
Orange II in orange I ... 211
Organic and ammoniacal nitrogen in fertilizers ... 18
Organic carbon in soils ... 4
Oxides of iron, aluminum, manganese, phosphorus and titanium in soils ... 6
Oxygen in potable water ... 407

Paints, white linseed oil, reagents used ... 73
Palm kernel oil in cacao butter and fat extracted from milk chocolate, detection 159
Paprika oil, iodine number ... 351

PAGE

- Paraffin in confectionery 387
- Paraffin and waxy substitutes in facing for tea 156
- Paris green 40
- Parr's table, density of carbon dioxide 556
- Peanut oil in oils, fats and waxes 330
- Pectin acid (di-galacfuronic acid) in fruits and fruit products 270
- Pentabromacetone, apparatus for drying by aspiration 274
- Pentosans, in grain and stock feed 284
    - vinegars 361
    - wines 142
- Peppermint extract 261
- Pepsin, in liquids 464
- Pepsin solution 464
- Peptone in meat 296
- Petroleum ether extract in coffee 152
    - leathers 93
    - tea 154
- Phenacetin 437, 444, 445
- Phenobarbital 484
- Phenol in caustic poisons 66
- Phenoldisulfonic acid method, nitrogen in potable water 405
- Phenolphthalein in chocolate preparations 467
    - tablets 466
- Phenolphthalein indicator 16, 55
- Phloroglucin 284
- Phosphoric acid, in ash of baking powders and baking chemicals 124
    - basic slag 27
    - beers 135
    - fertilizers 14
    - mineral water 418
    - roasted coffee 151
    - tea 154
    - vinegars 357
    - wines 139
- Phosphoric-pentoxide in eggs and egg products 248
- Phosphorus, in gelatin 303
    - meat and meat products 290, 300, 301
    - plants 110
    - soils 10
    - oxides of, in soils 6
- Phosphotungstic-phosphomolybdic acid 252
- Photomicrographs of malted milk and its allied products 232, 234
- Physical constants of 10% distillate, lemon and orange oils 258
- Physostigmine, threshold myotic dose 486
- Phytosterol in mixtures of animal and vegetable fats 327
- Pigments 184
    - in paints 76, 78, 79, 83
    - used for coloring or facing of tea 156
- Pilocarpine 450
    - microchemical identification 451

PAGE

- Pilocarpine, threshold myotic dose 486
- Pinene in lemon and orange oils 258
- Plants 102
- Platinic chloride solution 450
- Platinum solution 25
- Polariscopic constants, gelatin 304
- Polariscopic methods, sucrose, sugars and sugar products 367
- Polarization, of beers 135
  - honey 388
  - maple products 391
  - vinegars 360
- Polenske number, fats and oils 322
- Polenske value of cacao fat 162
- Potable water 403
- Potash in fertilizers 25
- Potassium, in baking powders and baking chemicals 124
  - fruits and fruit products 266
  - mineral water 415
  - plants 106
  - soap 53
- Potassium and sodium in soils 11
- Potassium chromate indicator 30, 173, 406
  - solution 26
- Potassium cyanide 51
- Potassium dichromate solution 358
- Potassium ferrocyanide 74
- Potassium hydroxide 16
  - alcoholic 459
- Potassium iodate 75, 450, 461
- Potassium permanganate solution 24, 46, 74, 141
- Potassium thiocyanate indicator 76
- Poultry and similar feeds, grit content 286
- Power-Chesnut method, caffeine in coffee 151
  - tea 155
- Prepared mustard 353
- Preservatives in beers 136
  - beverages and concentrates 132
  - butter 237
  - cream 227
  - fruits and fruit products 276
  - meat 295
  - meat extracts 302
  - milk 224
  - prepared mustard 354
  - vegetables and vegetable products 398
  - vinegars 362
  - wines 142
- Preservatives and artificial sweeteners 334
- Procaine hydrochloride 470
- Proctor and Searle method, mineral acidity of leathers 93

PAGE

Protein, in alimentary pastes........ 181
  beers........ 135
  bread........ 178
  dried and malted milk........ 230
  fruits and fruit products........ 270
  grain and stock feeds........ 279
  prepared mustard........ 354
  sweetened condensed milk........ 228
  tea........ 154
  wheat flour........ 167
  wines........ 142
Protein-nitrogen, in alimentary pastes........ 181
    white flour........ 169
Proteose, peptone and gelatin nitrogen in meat........ 296
Proteoses and gelatin in meat extracts........ 301
Pseudoephedrine, threshold mydriatic dose........ 486
Purée, microanalysis........ 400
Pyramidon........ 470

Quartz flour (silica), insecticides and fungicides........ 60
Quinine........ 441, 442, 452, 453, 454
  microchemical identification........ 451
  separation from strychnine........ 473
Quisumbing and Thomas, method........ 112
    table........ 528

Radioactivity, of foods and drugs........ 431
    standard solution........ 433
Radium, standard solution........ 431
Raffinose........ 373
Ramberg-Sjöström arsenic flask........ 477
Raw and spent materials, tanning materials........ 99
Reducing sugars, in beers........ 135
    beverages and concentrates........ 131
    honey........ 388
    maple products........ 391
    plants........ 112
    salad dressings........ 355
    tomato products........ 399
    vinegars........ 358
  other than dextrose........ 384
Refractive index........ 68, 161, 258, 315
Reichert-Meissl and Polenske values, cacao fat........ 162
      soluble volatile acids........ 322
Renovated butter and oleomargarine........ 238
Residual color in vanilla extract........ 254
Residue, ignited, in waters........ 404, 411
Resin oil in oils, fats and waxes........ 329
Resorcin test........ 390
Rice hulls in rice bran........ 286

PAGE
Riche and Bardy method, methyl alcohol in distilled liquors 146
Ricin solution 464
Robertson method, nitrate nitrogen in fertilizers 22
Roese-Gottlieb method, fat in cheese 243
  confectionery 386
  cream 225
  dried milk 230
  milk 217
Rosin in naval stores 68
  paints 78

Saccharin 338
Saccharometers, temperature, corrections to readings of 503
Salad dressings 355
Salicylates, total 446
Salicylic acid 334
  in presence of other phenols 468
Salol 445
Salt 428
  in butter 236, 237
    cheese 239
    grain and stock feeds 278
    prepared mustard 354
    method of reporting results 429
Salts, alkali, in soils 13
Sampling, drugs 347
Sand in plants 102
  tomato products 398
Sanglé-Ferrière method, abrastol (asaprol) 346
Santonica 472
Santonin 472
Saponification number of cacao fat 161
  fats and oils 321
Saturated and unsaturated fatty acids 324
Sausage and similar meat products, added water 289
Scales method, invert sugar 378
Schiff's reagent 148
Schmidt-Bondzynski method (modified), fat in cheese 239
Schönrock's table 510
Schultze's mixture 352
Schweitzer's reagent 352
Scopolamine, threshold mydriatic dose 486
Seed disinfectants, organic mercurial 62
Separation of natural coloring matters 194
  permitted coal tar food colors 188
Sesame oil in oils, fats and waxes 331
Silica in industrial water 422
  mineral water 413
  plants 102
  soils 5

PAGE
Silicotungstic acid solution ... 55
Silver, total, drugs ... 483
Silver nitrate solution ... 30, 111, 406
Silver proteinates ... 483
Silver sulfate in sulfuric acid, insecticides and fungicides ... 60
Sinibaldi method, abrastol (asaprol) ... 346
Sirups ... 363
Slag, Thomas or basic ... 27
Soap and soap emulsions ... 53, 54
Soda, alcoholic ... 174
Soda lime ... 116
Soda lye ... 55
Sodium, in baking powders and baking chemicals ... 124
  mineral water ... 415
  permitted food dyes ... 212
  plants ... 106
  soap ... 53
Sodium amalgam ... 58
Sodium and potassium cyanides ... 51
Sodium and potassium in soils ... 11
Sodium benzoate solution ... 450
Sodium cacodylate, arsenic content ... 478
Sodium carbonate fusion, soils ... 5
  in commercial coal tar food colors ... 211
  solution ... 252, 450
Sodium chloride in commercial coal tar food colors ... 198
  tomato products ... 399
  vegetables and vegetable products ... 397
Sodium hydroxide, alcoholic solution ... 73
  standard solution ... 28
  stock solution ... 24
Sodium iodide in commercial coal tar food colors ... 210
Sodium or potassium hydroxide solution ... 16
Sodium salicylate ... 443
Sodium sulfate in commercial coal tar food colors ... 200
Sodium thiosulfate ... 408
  solution for $SO_2$, standard, paints ... 75
  standard solution, paints ... 73
Soil reaction, qualitative test ... 11
Soils ... 1
Solids, in alimentary pastes ... 180
  beverages and concentrates ... 131
  commercial coal tar food colors ... 198
  cream ... 225
  eggs and egg products ... 244
  entire loaf of bread ... 177
  fruits and fruit products ... 265
  ginger extract ... 261
  industrial water ... 422
  leathers ... 93

PAGE

- Solids, in lemon and orange extracts 257
  - milk 214
  - mineral water 411
  - potable water 403
  - prepared mustard 353
  - roasted coffee 150
  - salad dressings 355
  - sugars and sugar products 363
  - sweetened condensed milk 228
  - tanning material extracts and liquors 96, 99
  - tomato products 398
  - unsweetened evaporated milk 227
  - vanilla extract 253
  - vinegars 357
  - wheat flour 165
  - wines 138
- Sörensen method, amino nitrogen in meat 300
- Soxhlet's modification of Fehling's solution 377
  - solution, factors to be used in connection with Lane-Eynon general volumetric method 526
- Soxhlet-Wein method, lactose 384
  - table, lactose 523
- Spearmint extract 261
- Specific gravity, of beers 134
  - beverages and concentrates 131
  - distilled liquors 143
  - lemon and orange extracts and oils 255, 257
  - milk 214
  - mineral water 411
  - oils, fats and waxes 314
  - paints 79
  - vanilla extract 251
  - vinegars 356
  - wines 136
- Spices, microscopic examination 351
- Spices and added pungent materials in vinegars 362
- Spices and other condiments 349
- Spores and yeast, tomato products 400
- Stanek's correction table 512
- Stannous chloride solution 306
- Starch, in baking powders and baking chemicals 121
  - cacao products 163
  - coffee 152
  - confectionery 385
  - fruits and fruit products 275
  - grain and stock feeds 282
  - meat 293
  - plants 114
  - spices and other condiments 350
  - wheat flour 172

PAGE

Starch indicator.... 35
Starch iodate paper, paints.... 75
Starch solution.... 74
Stems in tea.... 154
Stock feeds.... 277
Strontium in mineral water.... 414
Strychnine, in liquid preparations.... 474
 tablets.... 472
 microchemical identification.... 451
 separation from quinine.... 473
Sucrol or dulcin.... 346
Sucrose, in beverages and concentrates.... 131
 cacao products.... 158
 coffee.... 152
 confectionery.... 385
 fruits and fruit products.... 274
 grain and stock feeds.... 281
 honey.... 388
 lemon and orange extracts.... 257
 maple products.... 392
 plants.... 113
 salad dressings.... 355
 sugar beets.... 394
 sugars and sugar products.... 367
 sweetened condensed milk.... 229
 tomato products.... 399
 vanilla extract.... 253
 wines.... 138
Sucrose and raffinose.... 373
Sugar and dextrin in coffee.... 153
Sugar beets.... 394
Sugar in meat and meat extracts....294, 302
Sugar solutions, degrees Brix, specific gravity, and degrees Baumé.... 497
Sugars and sugar products.... 363
Sugars, in distilled liquors.... 145
 fruits and fruit products.... 274
 plants.... 112
 tanning material extracts.... 97
 vegetables and vegetable products.... 398
 wheat flour.... 167
 wines.... 138
Sulfanilic acid reagent.... 405
Sulfate, in paints.... 89
 salt.... 428
Sulfate sulfur in lime-sulfur solutions.... 59
Sulfates in vinegars.... 360
Sulfide sulfur, in lime-sulfur solutions.... 58
 paints.... 89
Sulfite-cellulose, detection, tanning material extracts.... 98
Sulfonated dyes, lower.... 208

PAGE

Sulfur, in commercial coal tar food colors ........ 205
  fruits and fruit products ........ 266
  lime-sulfur solutions ........ 57–59
  permitted food dyes ........ 212
  plants ........ 109
  soils ........ 9
  spices and other condiments ........ 351
Sulfur compounds in paints ........ 89
Sulfur dioxide, in gelatin ........ 304
  paints ........ 90
Sulfur, sulfide, in paints ........ 89
Sulfur, thiosulfate, in lime-sulfur solutions ........ 58
Sulfuric acid, determination of strength ........ 19
  in baking powder and baking chemicals ........ 124
    industrial water ........ 422
    mineral water ........ 415
    wines ........ 139
  reagent ........ 59
Sulfurous acid ........ 343
Suspended matter in potable water ........ 404
Sweetened condensed milk ........ 228
Sweetening substitutes in fruits and fruit products ........ 276

Tables, reference ........ 495
Tankage, bone content ........ 287
Tankage and bone, mechanical analysis ........ 14
Tannin, in leathers ........ 95
  spices and other condiments ........ 350
  tanning material extracts ........ 97
  tea ........ 155
  wines ........ 141
Tanning materials ........ 96
Tartaric acid, in baking powders and baking chemicals ........ 120
  beverages and concentrates ........ 131
  cheese ........ 240
  fruits and fruit products ........ 271
  vinegar ........ 361
  wines ........ 141
Tea ........ 154
Temperature corrections for polarization of sugars ........ 369
Thalleoquin test ........ 454
Thermometer, U. S. Bur. Standards tested, added water in milk ........ 222
Thinner for paints ........ 76
Thiosulfate, standard solution for lead arsenate ........ 44
Thiosulfate sulfur, lime-sulfur solutions ........ 58
Thomas or basic slag ........ 27
Thymol ........ 469
Tin in foods ........ 309
Titanium, oxide of, in soils ........ 6
  pigment, paints ........ 82

PAGE

Titer test for oils, fats and waxes ... 317
Tobacco and tobacco extract ... 55
Toluene distillation method, moisture, grain and stock feeds ... 277
    leathers ... 92
Toluol-insoluble matter in rosin ... 68
Tomato products ... 398
Tomato pulp, purée, sauce, paste, microanalysis ... 400
Tragacanth ... 475
Trillat method, methyl alcohol in distilled liquors ... 146
Tristearin in lard, detection ... 331
Turbidity of potable water ... 403
Turpentine oil ... 68

Unsaponifiable matter in eggs and egg products ... 248
Unsaponifiable residue, in alimentary pastes ... 181
    fats, oils and waxes ... 329
    wheat flour ... 170
Unsulfonated residue in mineral oils ... 53
    soap emulsions ... 55

Vanilla extract and its substitutes ... 251
Vanilla resins in vanilla extract ... 254
Vanillin in vanilla extract ... 251
Van Slyke method, amino nitrogen in meat ... 297
Vegetable tanned leather ... 92
Vegetables and vegetable products ... 397
Villavecchia test modified for sesame oil ... 331
Vinegars ... 356
Volatile oil in mustard seed ... 351
    tea ... 155
Volatile thinner, paints ... 76

Wagner's reagent ... 439
Water, in mineral oil soap emulsions ... 54
    paints ... 76
Water, added, in cream ... 225
    grape juice ... 276
    milk ... 219, 554
    sausage and similar meat products ... 289
  mineral ... 411
Water extract in tea ... 154
Water extractives in commercial coal tar food colors ... 207
Waters, brine and salt ... 403
Waxes ... 314
Waxes and fats in coffee ... 153
Wein method, maltose ... 383
  table, maltose ... 522
Wheat flour ... 165
Wichmann method, lead number of vanilla extract ... 253
Wijs method, iodine absorption number ... 321

PAGE
Wijs solution . . . . . . 73
Wiley method, melting point of fats and fatty acids . . . . . . 317
Wines . . . . . . 136
Wintergreen extract . . . . . . 261
Winton lead number, maple products . . . . . . 392
  method, lead number, vanilla extract . . . . . . 252
Wool dyeing procedure, coloring matters in foods . . . . . . 184
Wormseed, levant . . . . . . 472

Yeast and spores, tomato products . . . . . . 400

Zeiss butyro-refractometer readings . . . . . . 316
Zinc, in food . . . . . . 312
  gelatin . . . . . . 303, 304
  paints . . . . . . 87
  plants . . . . . . 108
  standard solution . . . . . . 411
Zinc arsenite . . . . . . 47
Zinc dust . . . . . . 21
Zinc oxide, in insecticides and fungicides . . . . . . 40
  paints . . . . . . 81
  zinc arsenite . . . . . . 47
Zinc sulfate solution . . . . . . 108

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